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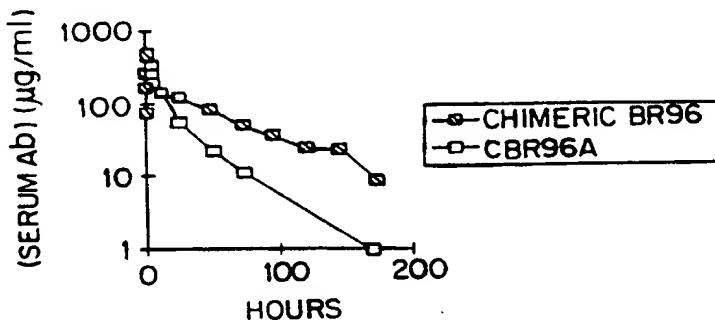


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(54) Title: A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS



(57) Abstract

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.

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5 **A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED
TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN
THERAPY AND IN VIVO DIAGNOSIS**

10 Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

15 **TECHNICAL FIELD OF THE INVENTION**

The present invention relates to methods for inhibiting or reducing immunoglobulin-induced toxicity resulting from therapy or in vivo diagnosis. Specifically, in lieu of using unmodified antibodies or recombinant binding proteins for in vivo use, the
20 invention provides the use of modified antibodies or recombinant binding proteins which have been structurally altered in the constant domain so that upon administration immunoglobulin-induced toxicity is reduced or inhibited.

BACKGROUND OF THE INVENTION

25

Over the years investigators have attempted to harness the immune system for therapeutic use. Immunoglobulin (Ig) molecules which constitute an important part of the immune system are of great interest because they (1) react with a diverse family of ligands, (2) possess different effector functions and (3) are of great
30 biological importance. Despite its potential, a persistent problem with

immunoglobulin immunotherapy has been, among other problems, the toxic effect to normal cells of using antibodies which recognize both normal and diseased cells. This problem is far-reaching because the majority of antibodies presently available recognize a target located on both normal and diseased cells (Slavin-Chiorini, et al.,
5 Int. J. Cancer 53: 97-103 (1993)).

The constant region can promote cell death through antibody dependent cell mediated cytotoxicity (ADCC) or by complement dependent cytotoxicity (CDC). Despite the deletion of portions of the constant region, particularly the CH₂ domain,
10 the antigen binding function can be retained (D. Yelton, M. Scharf, Mutant monoclonal antibody with alterations in biological functions, J. Exp. Methods 156:1131-1148 (1982)).

Others have generated a CH₂-deleted antibody (Mueller et al., Proc. Natl. Acad. Sci.
15 USA 87: 5702-5705 (1990)). Their findings provide that the CH₂-deleted antibody was cleared from the blood of tumor-bearing mice much faster than the corresponding intact antibody. Other in vivo findings also confirmed that a CH₂- deleted antibody, designated ch14.18DCH2, is a potentially useful reagent for radioimmunodetection of human tumors because of its reduced immunogenicity,
20 increased target specificity, and rapid clearance from circulation (Mueller et al.. Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990)).

Generally, whole antibody molecules are composed of two heavy (H) and two light (L) chains which are held together by covalent bonds (disulfide) and non-covalent interactions. Each chain contains a variable region (V) and a constant region (C).
25 The variable regions at the amino termini of the two chains form the antigen binding region. The constant region of the H chain has three components or domains. Occasionally, the first constant region domain (CH₁) interacts with the C region of the L chain through hydrophobic interactions and generally a disulfide bond,

depending on isotype. The next C region stretch is the hinge-acting disulfide bond stably introduced between two H chains. The second constant region domain (CH_2) is adjacent to the hinge region. CH_2 contains sequences important for effector functions of the antibody, such as the sequences responsible for complement fixation, and Fc receptor binding. The third constant region domain (CH_3) is located at the carboxyl terminus of the H chain, and is considered to play an important role in H chain assembly as well as some C region functions.

Today many antibodies in clinical trials are directed against tumor associated antigens. Most tumor associated antigens are not tumor specific but are also generally found on the cell surface of some normal, non-tumorigenic cells. The clinical use of some antibodies directed against tumor associated antigens are limited because of the toxicity associated with their use. Therefore, there is a need for methods for inhibiting toxicity associated with immunoglobulin use in the field of disease therapy (e.g., therapy for tumors, kidney disease, and the like) and in vivo diagnosis.

We addressed this need by discovering methods for inhibiting or reducing toxicity to normal cells generally associated with immunoglobulin immunotherapy or in vivo diagnosis, wherein the immunoglobulin recognizes both diseased and normal cells. Our discovery involves generating immunoglobulin molecules or Ig fusion proteins having structurally altered constant regions which inhibit or reduce immunoglobulin-induced toxicity.

25 SUMMARY OF THE INVENTION

The present invention provides methods for inhibiting immunoglobulin-induced toxicity by using known immunoglobulin or Ig fusion protein molecules which are structurally altered in their constant regions so that the resulting structurally altered

immunoglobulin or Ig fusion protein molecules exhibit reduced or inhibited toxicity in vivo compared to their original unmodified counterparts.

Structural alteration of the constant region may be effected in a number of ways as
5 long as it results in reducing or inhibiting immunoglobulin-induced toxicity.

- In accordance with the practice of the invention, structural alteration of the constant region is effected by deletion of the entire constant region. In another embodiment, only the CH₂ domain is deleted. In another embodiment, only that portion of the
10 CH₂ domain that binds the Fc receptor is deleted. In yet another embodiment, only that portion of the CH₂ domain that binds the complement component C1q is deleted. Alternatively, in another embodiment, multiple deletions in discrete Fc receptor and complement component binding domains are effected.
- 15 Alternatively, structural alteration is effected by single or multiple mutations in the CH₂ domain such as amino acid insertions and substitutions. The mutation or mutations must result in inhibiting immunoglobulin-induced toxicity. By way of example, the amino acids in multiple toxicity associated domains in the constant region can be altered so as to render the constant region unable to mediate a ADCC
20 response or activate complement thereby inhibiting immunoglobulin induced toxicity resulting from immunotherapy. Alternatively, multiple amino acids in a single toxicity associated domain in the constant region can be altered.

- Further alternatively, structural alteration can be effected by isotype switching
25 resulting in an altered immunoglobulin molecule that either does not induce toxicity or induces some limited toxicity but does not cause a harmful effect. For example, isotype switching can result in the constant region being unable to mediate a CDC or ADCC response or some other activity which mediates toxicity.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a line graph showing plasma clearance in high Le^y expressing dogs using chimeric BR96 versus constant region mutant of cBR96-2.

5

Figure 2 is a schematic diagram of a plasmid designated pTWD-cJVK.L1 including the chimeric (c)BR96-light chain (SEQ ID NO. 11).

Figure 3 is a schematic diagram of a plasmid designated pD16hJ1.L1 including the
10 human (h)BR96-light chain (SEQ ID NO. 13).

Figure 4 is a schematic diagram of a plasmid, designated pD17-hJm14-dCH2.H1, of hBR96-2A (i.e., human mutant BR96 having the H1, H2, and H3 mutations and the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

15

Figure 5 is a schematic diagram of a plasmid, designated pD17-cJ-dCH2.H1, of cBR96-A (SEQ ID NO. 10) (i.e., chimeric BR96 having the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

20 Figure 6 is a schematic diagram of a plasmid, designated pD17-cJ.H1, of cBR96.

Figure 7 is a line graph showing the results of an ELISA assay of (1) hBR96-2A-Dox to Le^y (closed diamond), (2) hBR96-2A to Le^y (96:0006A2 R/A)(closed square), (3) hBR96-2A to Le^y (96:0006B R/A)(closed triangle), and BR96-Dox to
25 Le^y (X).

Figure 8 is a line graph showing the results of an ELISA assay of (1) BR96-A-Dox to Le^y (closed diamond), (2) chiBR96 to Le^y (closed square), (3) cBR96-A to Le^y (96:0003 R/A)(closed triangle), and cBR96-Dox to Le^y (X).

Figures 9a-c are schematic diagrams showing the steps for deleting a CH₂ domain.

Figures 10a-c are schematic diagrams showing the construction of BR96 IgG1 CH₂
5 domain point mutations.

Figure 11 is a schematic diagram showing the construction of the pNg1/14 vector.

Figure 12 is a schematic diagram showing the construction of pD17-hBR96-2.

10

Figure 13 is a schematic diagram showing the construction of pD17-hJm14-dCH2.H1.

15

Figure 14 is the nucleic acid sequence of pD17-cJ-dCH2.H1, the plasmid shown in
Figure 5, chimeric BR96 having the CH₂ deletion.

Figure 15 is a line graph showing the results of an ELISA assay comparing whole
chiBR96 and deleted CH₂ chiBR96 on Le^y.

20

Figure 16 is a description of the seven structural alterations.

Figure 17 is a schematic diagram of a plasmid designated pD17-hG1b.

Figure 18 is the nucleic acid sequence of pD17-hJm14.H1.

25

Figure 19 is the nucleic acid sequence of pD17-hG1b.

Figure 20 is a line graph showing complement dependent cytotoxicity. In the
legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is

hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b mAb, negative control.

5 Figure 21 is a line graph showing antibody dependent cell-mediated cytotoxicity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b monoclonal antibody (mAb), negative control.

10

Figure 22 is a line graph showing binding activity of hBR96-2 constant region mutants on LeY-HSA. In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH₂ deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

15

Figure 23 is a line graph showing binding activity of hBR96-2 constant region mutants on LNFPIII-BSA. LNFPIII is a lacto-N-fucopentasose, a Lewis X trisaccharide with an additional lactose spacer (V Labs, Covington, LA). In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH₂ deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

20
25

Figures 24A and 24B provide a strategy for introducing multiple mutations by RPCR. (A) Diagram of the 1.4 kpb IgG heavy chain region showing the hinge CH₂ and CH₃ domains as boxed regions. Site-specific mutations to be introduced into CH₂ positions L1, L2, and L3 are encoded by complementary sets of mutant PCR

primers (A1 and A2; B1 and B2; and C1 and C2). The asterisks (*) indicate the number of amino acid changes introduced at each L position. The two PCR primers, Rs (Recombination -sense) and Ra (Recombination-antisense), flank the Eco-47-III restriction sites and mediate homologous recombination with vector ends. The 3' 5 ends of the oligonucleotides are represented by arrowheads. (B) A three-way homologous recombination event between fragments RsA2, A1Ra and the linearized vector produces the L1 mutant IgG. Two distally located sets of mutations (L1 and L2) are simultaneously introduced by increasing the number of recombining PCR produces as is shown in the four-way recombination of RsA2, A1B1, B1Ra with 10 vector.

Figure 25 is a gel showing Eco-47-III restriction endonuclease analysis of DNAs prepared from colonies generated by multiple PCR fragment RPCR. Lane M: 1kb ladder DNA marker (GIBCO/BRL Life Science Technology). Lanes 1-12: Twelve 15 randomly selected colonies resulting from quadruple homologous recombination events were used to prepare plasmid and digested with Eco47-III. Clones 1, 2, 6 and 9 contain the fully assembled 1.4 kpb insert.

Figure 26 provides the amino acid sequence for hBR96-2 heavy-chain variable 20 region and the human IgG1 constant region.

Figure 27 provides the amino acid sequence for hBR96-2A heavy-chain variable region and the human IgG1 constant region.

25 Figure 28 provides the amino acid sequence for chi BR96 heavy-chain variable region and the human IgG1 constant region without the CH₂ domain.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

5 As used herein the term "inhibiting immunoglobulin-induced toxicity" means to reduce or alleviate symptoms generally associated with toxicity caused by immunoglobulin or Ig fusion protein therapy, e.g., toxicity mediated by effector functions of the Fc receptor. For example, BR96 antibody recognizes and binds BR96 antigen which is found at some levels in the gastrointestinal tract and at
10 elevated levels in tumors (as compared to the gastrointestinal tract of normal tissues). The binding of BR96 antibody to BR96 antigen in vivo causes symptoms associated with gastrointestinal toxicity. These symptoms include rapid onset of vomiting, often with blood, and nausea. In humans the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach.

15

The pathology of the wound is limited and resolves. However, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity. For highly elevated levels of other antigens found in the central nervous system (CNS), liver, and other locations, the toxicity will be characterized by
20 symptoms other than those described above.

25

As used herein the term "immunoglobulin molecule" can be produced by B cells or be generated through recombinant engineering or chemical synthetic means. Examples of immunoglobulin molecules include (1) antibodies, e.g., polyclonal and monoclonal antibodies, chimeric or humanized, and (2) recombinant Ig containing binding proteins, e.g., Ig fusion proteins. Recombinant Ig containing binding proteins include cell surface proteins, e.g., CD antigens (in one embodiment, CTLA4), to which an Ig tail is joined.

As used herein the terms "structurally altered" or "structural alteration" means manipulating the constant region so that the resulting molecule or protein exhibits a diminished ability to induce toxicity. Structural alteration can be by chemical modification, proteolytic alteration, or by recombinant genetic means. Recombinant 5 genetic means may include, but is not limited to, the deletion, insertion and substitution of amino acid moieties.

As used herein the terms "multiple toxicity associated domains" means more than one discrete toxicity associated domain. As there appear to be at least two toxicity 10 associated domains in the immunoglobulin molecule, one roughly localized to amino acids 231-238 and another roughly localized to amino acids 310-331, an example of the structural alteration of multiple toxicity associated domains comprises the insertion, substitution or deletion of amino acid residues in both of these domains. This definition excludes structural alterations targeting a single toxicity associated 15 domain.

Merely by way of example, the constant region of the immunoglobulin molecule can be structurally altered so that the molecule no longer mediates a CDC or ADCC response. However, the methods of the invention encompasses the use of 20 structurally altered immunoglobulin molecules regardless of whether it mediates a CDC or ADCC response. The underlying requirement is that the altered molecule must inhibit immunoglobulin-induced toxicity.

Structural alteration can be effected in a number of ways. For example, structural 25 alteration can be effected by deletion of the entire constant region.

Alternatively, structural alteration can be effected by deletion of the entire CH₂ domain of the constant region. In this instance, deletion of the entire CH₂ domain may render the molecule unable to (1) bind an Fc receptor thereby eliminating the

molecule's possibility of mediating antibody-dependent cellular cytotoxicity (ADCC), (2) bind C1q, or (3) activate complement.

Alternatively, structural alteration can be effected by deletion of only that portion of
5 the CH₂ domain that binds the Fc receptor or complement.

Further alternatively, a single mutation or multiple mutations such as substitutions and insertions in the CH₂ domain can be made. The underlying requirement of any mutation is that it must inhibit, diminish, or block immunoglobulin-induced toxicity.

10 For example, this can be achieved by mutating the constant region such that the altered molecule is rendered unable to mediate a CDC response or an ADCC response, or to activate complement.

Alternatively, structural alteration can be effected by isotype switching (also known
15 as class switching) so that the altered molecule does not induce toxicity in the subject. In one embodiment, the constant region of the immunoglobulin is structurally altered so that it no longer binds the Fc receptor or a complement component, e.g., switching a molecule's original IgG isotype from IgG1 to IgG4. Isotype switching can be effected regardless of species, i.e., an isotype from a non-
20 human being can be switched with an isotype from a human being (E.D. Finkelman et al. (1990) Annu. Rev. Immunol. 8:303-333; T. Honjo et al. (1979) Cell 18: 559-568; T. Honjo et al. In "Immunoglobulin Genes" pp. 124-149 Academic Press, London)).

25 As used herein the term "Ig fusion protein" means any recombinantly produced antigen or ligand binding domain having a constant region which can be structurally altered.

As used herein "cytotoxic agent" includes antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents. Specific examples within these groups include but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, 5 tenoposide, vincristine, vinblastine, colchicine, supporin, gelonin, PE40, bryodin, dihydroxy anthracin dione, actinomycin D, and 1-dehydrotestosterone.

As used herein the term "BR96" refers to (1) the whole BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, (2) chimeric BR96 10 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, or (3) BR96 mutant molecules disclosed in PCT No. 95/305444, published March 6, 1996.

As used herein, "treating" means to (1) provide tumor regression so that the tumor is 15 not palpable for a period of time (standard tumor measurement procedures may be followed (A.B. Miller et al. "Reporting results of cancer treatment" Cancer 47:207-214 (1981)); (2) stabilize the disease; or (3) provide any clinically beneficial effects.

As used herein, an "effective amount" is an amount of the antibody, 20 immunoconjugate, or recombinant molecule which kills cells or inhibits the proliferation thereof.

As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular or 25 subcutaneous administration, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

As used herein, "pharmaceutically acceptable carrier" includes any material which when combined with the antibody retains the antibody's specificity or efficacy and is

non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including
5 coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may
10 also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

As used herein, "mutation" means a single amino acid or nucleic acid mutation or multiple mutations by whatever means, e.g., homologous recombination, error prone
15 PCR, or site directed mutagenesis.

In order that the invention herein described may be more fully understood, the following description is set forth.

20 METHODS OF THE PRESENT INVENTION

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from the use of immunoglobulin during therapy or in vivo diagnosis. For example, the methods of the invention would be useful to minimize
25 the toxicity associated with prolonged clinical exposure to immunoglobulin use during or after tumor imaging with radiolabeled antibodies.

In accordance with the practice of this invention, the subject includes, but is not limited to, human, equine, porcine, bovine, murine, canine, feline, and avian

subjects. Other warm blooded animals are also included in this invention.

This method comprises administering an immunoglobulin molecule to the subject. The immunoglobulin can be IgG, IgM, or IgA. IgG is preferred.

5

In one embodiment of the invention, the immunoglobulin molecule recognizes and binds Le^y. In another embodiment, the immunoglobulin recognizes and binds Le^x. In a further embodiment, the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma deposited on February 22, 1989 with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and accorded ATCC Accession No.: HB 10036. In yet another embodiment, the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma deposited on May 23, 1990, with the ATCC, 12301 Parklawn Drive, Rockville, MD 20852 and accorded ATCC Accession No.: HB 10460.

15

In accordance with the practice of the invention, the immunoglobulin can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC binds. Also, in accordance with the practice of the invention, the immunoglobulin can be an anti-idiotypic antibody.

20
25

As required by the invention, at least a portion of the constant region of the immunoglobulin molecule is structurally altered. Structural alteration can be effected by a number of means. In one embodiment, the entire constant region, i.e., CH₁, CH₂, and CH₃ domains, can be deleted.

In another embodiment, only the CH₂ domain is deleted from the immunoglobulin molecule (e.g., cBR96-A (Figure 5), hBR96-2A (Figure 4). In this embodiment, the

CH₂ deletion may result in a molecule unable to bind the Fc receptor or a complement component.

In another embodiment, only that portion of the CH₂ domain which binds the 5 complement component C1q is deleted. In yet another embodiment, mutations in specific portions of the CH₂ domain are made. For example, the immunoglobulin molecule may be modified by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited.

A discussion of such mutations are further found hereinafter.

10

Regardless of the means, the underlying requirement for any structural alteration of the constant region is that immunoglobulin-induced toxicity is substantially reduced or inhibited. In one embodiment, immunoglobulin-induced toxicity is inhibited by structurally altering the constant region such that the molecule's ability to mediate a

15 CDC response or ADCC response and/or activate the complement cascade is prevented or inhibited. Methods for determining whether the molecule is able to inhibit a CDC response are well known, e.g., one method involves a ⁵¹Cr-release test (H. Garrigues et al. Int. J. Cancer 29:511 (1982); I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to inhibit an ADCC 20 response are well known (I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to activate a complement cascade are well known.

In another embodiment of the invention, the method comprises administering to the 25 subject an Ig fusion protein having a structurally altered constant region. Structural alteration of the constant region may include deletion of the entire C region or portions thereof, e.g., alteration of the CH₂ domain so that the altered molecule no longer binds the Fc receptor or a complement component.

The invention further provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject. The method comprises administering to the subject an antibody which has been modified so that at least a portion of the constant region has been structurally altered as discussed supra. In one embodiment, the antibody recognizes and binds Le^y. In another embodiment, the antibody recognizes and binds to Le^x.

In accordance with the practice of this invention, the antibody can be monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC. Alternatively, the antibody can be chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC. Further, the antibody can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC binds.

Additionally, the present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject. The disease will vary with the antigen sought to be bound. Examples of diseases include but are not limited to immunological diseases, cancer, cardiovascular diseases, neurological diseases, dermatological diseases or kidney disease.

This method comprises the following steps. Step one provides selecting an antibody for a target. Generally, the target is associated with the disease and the antibody directed to the target is known. For example, the target can be the BR96 antigen and the antibody selected is BR96.

Step two of this method provides structurally altering the constant region of the antibody so selected so that immunoglobulin induced toxicity is inhibited. Inactivation can include any of the means discussed above. For example, inactivation can be effected by structurally altering multiple toxicity associated 5 domains in the CH₂ domain of the constant region of the Ig protein so selected.

Step three of this method provides administering the structurally altered antibody of step two to the subject under conditions that the structurally altered antibody recognizes and binds the target and that such binding directly or indirectly alleviates 10 symptoms associated with the disease.

In accordance with the invention, in one embodiment step one provides selecting an Ig fusion protein for a target. Further, the method provides mutating the Ig fusion protein so selected by structurally altering the CH₂ domain of the constant region of 15 the Ig protein by the same means discussed above.

The invention further provides methods to treat human carcinoma. For example, the immunoglobulin, antibody, or Ig fusion protein discussed above can be used in combination with standard or conventional treatment methods such as 20 chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma.

Techniques for conjugating therapeutic agents to immunoglobulins are well known 25 (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellström et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In

Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

5

Alternatively, the structurally altered antibody or Ig fusion protein can be coupled to high-energy radiative agents, e.g., a radioisotope such as ¹³¹I; which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order,

- "Analysis, Results, And Future Prospective Of The Therapeutic Use Of
10 Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985)). According to yet another embodiment, the structurally altered BR96 antibody can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

15

Still other therapeutic applications for the structurally altered antibody or Ig fusion protein of the invention include conjugation or linkage, e.g., by recombinant DNA techniques or protein chemical techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in

- 20 combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects Of Antibody-alkaline Phosphatase", Proc. Natl. Acad. Sci. USA, 85:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activities of Phosphorylated Mitomycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates",
25 Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," FASEB J. 4:188-193 (1990)).

It is apparent therefore that the present invention encompasses pharmaceutical compositions including immunoglobulin molecules, antibodies, and Ig fusion proteins all having structurally altered CH₂ domains, and their use in methods for treating human carcinomas. For example, the invention includes pharmaceutical compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of a structurally altered BR96 and a pharmaceutically acceptable carrier.

The compositions may contain the structurally altered antibody or Ig fusion protein or antibody fragments, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The compositions of the invention can be administered using conventional modes of administration including, but not limited to, intrathecal, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The composition of the invention can be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

In accordance with the practice of the invention, the pharmaceutical carrier can be a lipid carrier. The lipid carrier can be a phospholipid. Further, the lipid carrier can be a fatty acid. Also, the lipid carrier can be a detergent. As used herein, a detergent 5 is any substance that alters the surface tension of a liquid, generally lowering it.

In one example of the invention, the detergent can be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for 10 example Triton WR-1339 and Triton A-20).

Alternatively, the detergent can be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

15 Additionally, in accordance with the invention, the lipid carrier can be a liposome. As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

20 The most effective mode of administration and dosage regimen for the compositions of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician.

The interrelationship of dosages for animals of various sizes and species and humans based on mg/m² of surface area is described by Freireich, E.J., et al. Cancer 25 Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses can be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses can be

administered daily or proportionally reduced depending on the specific therapeutic situation).

THE MOLECULES OF THE INVENTION

5

The present invention provides structurally altered BR96 or BR96 Ig fusion proteins. Structurally altered BR96 antibodies or Ig fusion proteins have the variable region of BR96 and a modified constant region. This modification provides structurally altered BR96 antibodies or Ig fusion proteins with the ability to inhibit 10 immunoglobulin-induced toxicity.

Various embodiments of structurally altered BR96 or BR96 Ig fusion proteins have been made.

- 15 In one embodiment, designated cBR96-A, the entire CH₂ domain of cBR96 was deleted. CBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 10. cBR96 is expressed by a plasmid having the sequence in SEQ ID NO. 9.
- 20 In another embodiment, designated hBR96-2A, the entire CH₂ domain of hBR96 was deleted. hBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 12. hBR96 is a mutant BR96 having the H1, H2, and H3 mutations described in PCT Application No. 95/305444, published March 6, 1996.
- 25 In yet another embodiment, designated hBR96-2B, the leucine residue located at amino acid position 235 is mutated to alanine. Additionally, the glycine residue located at amino acid position 237 is mutated to alanine. The amino acid position numbering used is described in Kabat et al. Sequences of Proteins of Immunological Interest 5th Edition (1991) United States Department of Health and Human Services.

In a further embodiment, designated hBR96-2C, the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine
5 using standard protocols (Alexander R. Duncan and Greg Winter "The binding site for C1q on IgG" Nature 332:738 (1988)).

- In another embodiment, designated hBR96-2D, the proline residue at position 331 is mutated to alanine (M-H. Tao et al., "Structural features of human immunoglobulin
10 G that determine isotype-specific differences in complement activation" J. Exp. Med. 178:661-667 (1993); Y. Xu et al., "Residue at position 331 in the IgG1 and IgG4 domains contributes to their differential ability to bind and activate complement" J. Biol. Chem. 269:3469-3474 (1994)).
15 In an additional embodiment, designated hBR96-2E, the leucine residue at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine (A. Morgan et al., "The N-terminal end
20 of the CH₂ domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc(gamma)RI and Fc(gamma)RIII binding" Immunol. 86:319-324 (1995)).

In yet a further embodiment, designated hBR96-2F, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine;
25 and the proline residue located at position 331 is mutated to alanine.

In yet another embodiment, designated hBR96-2G, the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is

mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

In another embodiment, designated hBR96-2H, the leucine residue located at 5 position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

10

Depending on its form, a structurally altered BR96 antibody or fusion protein can be a monofunctional antibody, such as a monoclonal antibody, or bifunctional antibody, such as a bispecific antibody or a heteroantibody. The uses of structurally altered BR96, i.e., as a therapeutic or diagnostic agent, will determine the different forms of 15 structurally altered BR96 which is made.

Several options exists for antibody expression. Immunoexpression libraries can be combined with transfectoma technology, i.e., the genes for the Fab molecules derived from the immunoglobulin gene expression library can be connected to the 20 desired constant-domain exons. These recombinant genes can then be transfected and expressed in a transfectoma that would secrete an antibody molecule.

Once produced, the polypeptides of the invention can be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Such 25 derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the BR96 antigen or portions thereof.

It is a well-established principle of protein chemistry that certain amino acid

substitutions, entitled "conservative amino acid substitutions," can frequently be made in a protein without altering either the conformation or the function of the protein.

- 5 Amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid

- 10 (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional

- 15 structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V).

Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R)

- 20 are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

- 25 In one embodiment of the present invention, the polypeptide is substantially pure, i.e., free of other amino acid residues which would inhibit or diminish binding of the polypeptide to its target and would inhibit or reduce gastrointestinal toxicity which are normally exhibited during or after antibody therapy.

NUCLEIC ACID MOLECULES ENCODING THE PRESENT INVENTION

The nucleotide sequences and the amino acid sequences of the variable and constant regions of BR96 are known. The sequence for the immunoglobulin constant region
5 is known and provided in Figure 18. Specific mutations in the constant region of the BR96 antibody were made. Nucleic acid molecules encoding the seven mutants described above (hBR96-2B through hBR96-2H) are as follows.

In hBR96-2B, alanine at amino acid positions 235 and 237 is encoded by codons
10 GCU, GCC, GCA, or GCG.

In hBR96-2C, serine at positions 318, 320, and 322 is encoded by UCU, UCC,
UCA, or UGG.

15 In hBR96-2D, alanine at position 331 is encoded by codons GCU, GCC, GCA, or
GCG.

In hBR96-2E, alanine at positions 235 and 237 is encoded by codons GCU, GCC,
GCA, or GCG. Serine at positions 318, 320, and 322 is encoded by UCU, UCC,
20 UCA, or UGG.

In hBR96-2F, alanine at positions 235, 237, and 331 is encoded by codons GCU,
GCC, GCA, or GCG.

25 In hBR96-2G, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or
UGG. Further, the alanine at position 331 is encoded by codons GCU, GCC, GCA,
or GCG.

In hBR96-2H, alanine at positions 235, 237, and 331 is encoded by codons GCU,

GCC, GCA, or GCG. Additionally, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG.

Any of the above can be deoxyribonucleic acid (DNA), e.g., complementary DNA
5 (cDNA), or ribonucleic acid (RNA).

IMMUNOCONJUGATES

Immunoconjugates (having whole antibody or Ig fusion proteins) may be
10 constructed using a wide variety of chemotherapeutic agents such as folic acid and anthracyclines (Peterson et al., "Transport And Storage Of Anthracyclines In Experimental Systems And Human Leukemia", in Anthracycline Antibiotics In Cancer Therapy, Muggia et al. (Eds.), p. 132 (Martinus Nijhoff Publishers (1982); Smyth et al., "Specific Targeting of Chlorambucil to Tumors With the Use of
15 Monoclonal Antibodies", J. Natl. Cancer Inst., 76:503-510 (1986)), including doxorubicin (DOX) (Yang and Reisfeld "Doxorubicin Conjugated with a Monoclonal Antibody Directed to a Human Melanoma-Associated Proteoglycan Suppresses Growth of Established Tumor xenografts in Nude Mice PNAS (USA)"
85:1189-1193 (1988)), Daunomycin (Arnon and Sela "In Vitro and in vivo Efficacy
20 of Conjugates of Daunomycin With Anti-Tumor Antibodies" Immunol. Rev., 65:5-27 (1982)), and morpholinodoxorubicin (Mueller et al., "Antibody Conjugates With Morpholinodoxorubicin and Acid-Cleavable Linkers", Bioconjugate Chem., 1:325-330 (1990)).

25 BR96 has been conjugated to doxorubicin and has been shown to be effective in therapy of certain cancers or carcinomas (Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Casazza, A.M., Firestone, R.A., Hellström, I., and Hellström, K.E. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science, 261:212-215, 1993).

In accordance with the practice of the invention, structurally altered BR96 can be used in forms including unreduced IgG, reduced structurally altered IgG, and fusion proteins (PCT Application No. 95/305444, published March 6, 1996).

5

Suitable therapeutic agents for use in making the immunoconjugate includes Pseudomonas exotoxin A (PE) in either the native PE or LysPE40 form. LysPE40 is a truncated form containing a genetically modified amino terminus that includes a lysine residue for conjugation purposes. Doxorubicin is also a suitable therapeutic agent.

10

Additional examples of therapeutic agents include, but are not limited to, antimetabolites, alkylating agents, anthracyclines, antibiotics, and anti-mitotic agents.

15

Antimetabolites include methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine.

20

Alkylating agents include mechlorethamine, thiopeta chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin.

25

Anthracyclines include daunorubicin (formerly daunomycin) and doxorubicin (also referred to herein as adriamycin). Additional examples include mitozantrone and bisantrene.

Antibiotics include dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC).

Antimitotic agents include vincristine and vinblastine (which are commonly referred to as vinca alkaloids).

- 5 Other cytotoxic agents include procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons.

Further examples of cytotoxic agents include, but are not limited to, ricin, bryodin, gelonin, supporin, doxorubicin, taxol, cytochalasin B, gramicidin D, ethidium
10 bromide, etoposide, tenoposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, and glucocorticoid.

- Clearly analogs and homologs of such therapeutic and cytotoxic agents are encompassed by the present invention. For example, the chemotherapeutic agent
15 aminopterin has a correlative improved analog namely methotrexate.

- Further, the improved analog of doxorubicin is an Fe-chelate. Also, the improved analog for 1-methylnitrosourea is lomustine. Further, the improved analog of vinblastine is vincristine. Also, the improved analog of mechlorethamine is
20 cyclophosphamide.

METHODS FOR MAKING MOLECULES OF THE INVENTION

- There are multiple approaches to making site specific mutations in the CH₂ domain
25 of an immunoglobulin molecule. One approach entails PCR amplification of the CH₂ domain with the mutations followed by homologous recombination of the mutated CH₂ into the vector containing the desired immunoglobulin, e.g., hBR96-2. For example, hBR96-2B and hBR96-2D have been made by this method.

Another approach would be to introduce mutations by site-directed mutagenesis of single-stranded DNA. For example, vector pD17-hG1b, which contains only the constant region of IgG1 and not the V domain of hBR96, has the f1 origin of replication. This gives the vector the properties of a phagemid and site-directed 5 mutagenesis experiments can be performed according to the methods of Kunkel, et al. (Kunkel, T.A., J.D. Roberts, and R.A. Zakour, 1987 Methods Enzymol. 154:367-383) as provided in the Bio-Rad Muta-Gene® phagemid *in vitro* mutagenesis kit, version 2. For example, hBR96-2B, -C, -D, -E, -F, -G, and -H were made by this method.

10

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

15

EXAMPLE 1

The following standard ELISA protocol was used.

20 **Materials:** Immulon2 96 well plates and Genetic Systems Specimen Diluent Concentrate (10x); antibody conjugate was Goat Anti Human Kappa-HRP Mouse Adsorbed, Southern Biotech. at 1:10,000 in Genetic Systems Conjugate Diluent (1x); Genetic Systems EIA Chromogen Reagent (TMB) (1:100); Genetic Systems EIA Buffered Substrate (1x); primary antibody or antigen were AffiniPure F(ab')₂ 25 Fragment Goat Anti Human IgG Fc Fragment specific (Jackson Immuno Research), Goat Anti Human Kappa-UNLB (Southern Biotechnology Associates), Le^y-HSA (Alberta Research Council).

Methods: Dilute primary antibody or antigen to 1.0 µg/ml in 0.05M Carb/Bicarb buffer. Add 100µl of the diluted solution per well in Immulon 2 plates. Seal plates and incubate O.N. at 4°C.

5 Block plates by flicking them and blotting on paper towels. Add 200µl/well of Genetic Systems, Specimen Diluent Concentrate (1x). Incubate at least 1 hour at room temperature and then dump the contents of the plates. Wash the plates 3x in saline/Tween. Blot to dry. Allow the plates to dry at R.T. (45 min. to 1 hour). Seal and store the plates at 4°C.

10

Test samples as follows. Dilute samples and standards in Specimen Diluent at 1:10. Perform serial dilutions in separate round bottom plates. Transfer 100µl/well of final dilutions to antigen coated assay plates; then incubate O.N. at 4°C. Wash plates 3x with saline/Tween.

15

For conjugation add 100 µl/well of antibody-HRP conjugate in Genetic Systems Conjugate Diluent (1x). Incubate plates at Room Temp. for 60 min. Wash plates 3x in saline/Tween.

20 Add 100 µl/well of Genetic Systems EIA Chromogen Reagent (TMB) 1:100 in EIA Buffered Substrate (1x). Incubate at R.T. for 15 min. and stop with 1N H₂SO₄ 100 µl/well. Read plate at 450/630nm in EIA plate reader.

EXAMPLE 2

25

Construction of CH₂ deleted BR96 molecules

Strategy for Deleting CH₂ Domains: To construct CH₂ deleted BR96 molecules, the hinge, CH₂ and CH₃ domains were removed from chimeric BR96 and humanized

BR9696-2 IgG1 molecules by an Eco47-III restriction digestion in non-coding regions. The hinge and CH₃ domains were amplified by polymerase chain reaction (PCR) from a human IgG1 (pN γ 1.14) molecule lacking the CH₂ domain. Two oligonucleotides (Sense 49mer, Antisense 50mer) homologous to the sequences of 5 IgG1 constant region at both sides preserving E.co47-III sites were synthesized. The amplified hinge and CH₃ domain PCR fragments were added into Eco47-III sites on BR96 IgG1 molecules by in vivo homologous recombination (P. Bubeck et al., Nucleic Acid Research (1993) 21:3601-3602). The new BR96 IgG1 molecules were verified by restriction mapping and sequencing.

10

A sewing PCR strategy was used for the construction of CH₂ deleted human IgG1 (pN γ 1.14) (Robert M. Horton, et al. (1990) Biotech 8 (5)P, 528).

The CH₁ domain was amplified as a 580 bp fragment with a sense oligonucleotide 15 (5' TGG CAC CGA **AAG CTT** TCT GGG GCA GGC CAG GCC TGA 3') (primer A) and an antisense oligonucleotide (5' **TCC GAG CAT GTT GGT ACC CAC GTG GTG GTC GAC** GCT GAG CCT GGC TTC GAG CAG ACA 3') (primer B) from a linearized human IgG1 constant region vector (pN γ 1.7). The PCR fragment extends from the 5' end of the Hind-III site (in bold) through the Cel-II, Sal-I, Dra- 20 III, Kpn-I, 6 bp nucleotide spacer and Mro-I sites (in bold) at the 3' end of the CH₁ domain.

The CH₃ domain was then partially amplified (to the Xba-I site) with a sense primer (5' **GTC GAC CAC CAC GTG GGT ACC AAC ATG TCC GGA GCC ACA TGG ACA GAG GCC GGC T** 3') (primer C) and an antisense primer (5' CTG GTT CTT GTT CAT CTC CTC **TCT AGA TGG** 3') (primer D) from a linearized human IgG1 constant region vector (pN γ 1.7). A PCR fragment (about 150 bp) with Sal-I, Dra-III, Kpn-I, 6 nucleotide spacer and Mro-I sites (in bold) on its 5' end, extends only through the Xba-1 site (in bold) within the CH₃ domain.

The CH₁ and CH₃ partial PCR fragments were combined in a PCR without any primer. The reaction was run through two full cycles of denaturation and re-annealing to allow the fragments to combine at the homologous region at the 3' ends. Primers A and D (described above) were added to the reaction and the PCR cycle was completed. The polymerase extends the DNA with primer A and primer D, yielding a full-length (660 bp) PCR fragment. The newly extended PCR fragment is arranged from the 5' end to the 3' end in the following order: Hind-III - CH₁ - Cel-II - Sal-I - Dra-III - Kpn-I - 6 bp spacer - Mro-I - CH₃ partial - Xba-1.

10

The combined PCR fragment, with the CH₁ and partial CH₃ domains, was then cloned by a blunt end ligation into a Sma-I site on a pEMBL18 vector and the sequence was confirmed by dideoxy sequencing (Sanger et al. (1977) PNAS (USA) 74:5463-5466).

15

To transfer the CH₁ and partial CH₃ into a mammalian expression vector, both the pEMBL18 and pN γ 1.7 vectors were digested with Hind-III and Xba-I. The Hind-III and Xba-I fragment was ligated into the same sites on a linearized pN γ 1.7 vector. The new construct, with CH₁ and a full CH₃ domain, was designated the pN γ 1.10 vector.

20

The hinge fragment was amplified from a Hind-III digested pN γ 1.7 vector with the primers designed to flank the hinge exon with a Sal-I and a Dra-III cloning site at each end. These sites also exist between the CH₁ and CH₃ domains of the pN γ 1.10 construct. The sense oligonucleotide (5' ACC ATG **GTC GAC** CTC AGA CCT GCC AAG AGC CAT ATC 3') with a 6 bp spacer and a Sal-I cloning site (in bold) and the antisense oligonucleotide (5' CAT GGT **CAC GTG** GTG TGT CCC TGG ATG CAG GCT ACT CTA G 3') with a 6 bp spacer and a Dra-III cloning site (in bold) were used for the amplification of the hinge fragment (250 bp).

The hinge region PCR fragment was cloned into a Sma-I site on pEMBL18 by blunt end ligation. Both the pEMBL18 with the hinge domain and the pN γ 1.10 with the CH₂ and CH₃ domains were digested with Sal-I and Dra-III. The digested hinge 5 fragment was cloned into the Sal-I and Dra-III linearized sites on the pN γ 1.10 vector. The new construct, now carrying the CH₁, hinge and CH₃ domains, was designated pN γ 1.11.

To make the final CH₂ deleted human IgG1 construct, both the pN γ 1.11 construct 10 and pN γ 1.11 vector were digested with BamH1 and HindIII. A fragment containing the CH₁, hinge and CH₃ domains was cloned into the linearized pN γ 1.11 vector. The new constant region IgG1 construct lacks the CH₂ domain and is designated pN γ 1.14 (Figure 11).

15 For digestion of BR96 IgG1 with Eco47-III, a restriction fragment with hinge, CH₂ and CH₃ domains was identified on the constant region sequence of BR96 IgG1 vector in both chimeric and humanized molecules. The 5' end of this fragment lies inside the intron between CH₁ and hinge and the 3' end is located inside the CH₃ intron of the BR96 IgG1 molecule. The hinge, CH₂ and CH₃ domains (1.368 kb 20 fragment) were removed from BR96 IgG1 molecules by Eco47-III restriction digestion. The Eco47-III is a blunt end cutter. The BR96 IgG1 DNA digested with this enzyme does not require any pretreatment before cloning. Figure 12 is a diagrammatic representation of the pD17-hBR96-2 vector showing the Eco47-III sites used in cloning.

25

The CH₂ deleted BR96 IgG1 was then constructed as follows. The hinge and CH₃ domains were amplified from a CH₂ deleted L6 IgG1 (pN γ 1.14) construct with a sense oligonucleotide (5'

CAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTGACCTCAG

A 3') homologous to the constant region sequence of IgG1 at the 5' end of the Eco47-III site (in bold) and an antisense oligonucleotide

(5'GGAAAGAACCATCACAGTCTCGCAGGG

CCCAGGGCAG**CGCTGGGTGCTT** 3') homologous to the constant region

5 sequence of IgG1 at the 3' end of the Eco47-III site (in bold). The Eco47-III site at the 3' end of the pN γ 1.14 construct is modified in the cloning process. The Eco47-III site is thus introduced into an antisense primer and used in amplification of the hinge and CH₃ domains.

10 The pD17-BR96 IgG1 vector was digested with Eco47-III and the hinge, CH₂ and CH₃ domains were removed. The linearized pD17-BR96 IgG1 vector was mixed with equimolar amounts of hinge and CH₃ PCR fragments. Cotransformation of the PCR fragment with linearized DNA into E.coli DH5a competent cells resulted in a recombinant molecule, mediated by homologous recombination in bacteria. This
15 construct lacks the CH₂ domain of BR96 IgG1 molecules, and is designated pD17-BR96-dCH2 (Figure 13).

1.9 grams of CH₂-deleted chimeric BR96 was obtained as raw material from 89L of culture supernatant.

20

EXAMPLE 3

Toxicity, localization and clearance of CH₂-deleted chimeric BR96 was tested in vivo as follows.

25

Three dogs received 400 mg/m² of cBR96-A, the CH₂ deletion mutant of chimeric BR96, and two received chimeric BR96. Both molecules had been mildly reduced and alkylated. This is required to prevent dimerization of the deletion mutant into a tetravalent form. Both control dogs experienced the typical GI toxicity and none of

the three receiving the mutant displayed any toxicity. The control dogs and two of the test dogs were sacrificed at 1 hr to obtain duodenal tissue to measure antibody localization. Both control dogs had grossly visible GI pathology, and the test dogs had normal appearing GI tissue. The third dog has continued to show no signs of
5 toxicity.

Results: A significant amount of localization of the CH₂ deleted cBR96 (cBR96-A) occurred to the GI tract in dogs treated with 400 mg/m², although the intact chiBR96 localized slightly better. The levels of localization indicate that roughly equivalent
10 amounts of intact and CH₂ deleted cBR96 was delivered to the GI tract in these dogs.

Table 5. Localization of cBR96 to GI tissue.

Group	Animal	Specific	mean
Localization			
cBR96	#271	155	
			135
	#272	114	
cBR96-A	#273	126	
			89
	#274	52	

15

Using the mean level of specific localization, an amount of cBR96-A equivalent to at least 66% of the amount of cBR96 was delivered to the target organ of toxicity, the duodenum. Based on the dose ranging done with cBR96 in dogs (some clinical
20 signs of toxicity seen at doses of 10 mg/m²), even if this difference is real, it could

not explain the difference between significant toxicity and no toxicity, evaluation to date indicated that dogs treated with cBR96-A had no toxicity, pending microscopic histopathologic examination. This evaluation was based on analysis of 2 frozen blocks per dog and 2 sections per block. Replicates were quite good. We also ran 5 historical frozen tissues from dogs treated with native cBR96 or F(ab)2/BR96 and the levels of localization for those tissues were 110 and 0, respectively, consistent with our previous data.

Assuming that there is no toxicity at marginally higher (2X) doses of cBR96-A, 10 these data indicate that the CH₂ domain is associated with the induction of acute gastroenteropathy, and that the removal of this domain prevents the induction of gastroenteropathy mediated by BR96.

This study confirms the results showing that F(ab')2 is not toxic in the dog model 15 and that the toxicity is mediated by the constant region. The CH₂ deletion mutant is a candidate for targeting agents clinically. Because of the very long half-life of chimeric BR96, some decrease in the mutant's half-life should be acceptable.

Figure 1 shows the measurement of the clearance of the cBR96-A in high Le^Y 20 expressing dogs. The study used chimeric versus constant region mutant of cBR96-2.

CBR96-2 did clear faster than the chimeric BR96. The localization of cBR96-A to the gastrointestinal epithelium is not significantly affected by this more rapid 25 clearance. More than enough of the cBR96-A localized to have caused toxicity.

Discussion: The constant region of chimeric IgG is responsible for the GI toxicity seen in clinical trials, e.g. with chiBR96-dox. The GI toxicity seen in the dog model is very similar to the clinical toxicity. Both in man and dog, administration of the

unconjugated antibody mediates an acute GI toxicity characterized by rapid onset of vomiting, often with blood.

In man the bleeding is limited to the fundus of the stomach, causing erosion of the
5 superficial mucosa of the stomach. Although the pathology of the wound is limited and resolves, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity.

This toxicity is mediated in man and dog by the antibody molecule alone. At higher
10 doses of the antibody-dox conjugate, additional toxicity is seen in the dog model, probably due to doxorubicin. Although the intact IgG of BR96 causes toxicity in dog and man, the F(ab')2 molecule (divalent and lacking only in the constant region) is not toxic in dogs. This finding has motivated our attempts at high levels, and improves the affinity and specificity of BR96 for tumor antigen.

15

The CH₂ domain is known to mediate complement and FcR binding. It was not known that structural alteration of the CH₂ domain would result in immunoglobulin-induced toxicity inhibition.

20 Toxicology study of hBR96-2B

The toxicology study of hBR96-2B in high Lewis Y expressor dogs (n=2) showed that a dose of 400 mg/m² did not cause hematemesis nor bloody stools, in contrast to BR96 which consistently causes one or both signs. A dog sacrificed at 24 hrs had
25 normal gross appearance of the GI tract, again in marked contrast to chimeric BR96 which causes hemorrhagic lesions and mucosal erosions.

EXAMPLE 4

The polymerase chain reaction (PCR) is a widely used and versatile technique for the amplification and subsequent modification of immunoglobulin genes. The 5 rapidity and accuracy with which antibody genes can be modified in vitro has produced an assortment of novel antibody genes can be modified in vitro has produced an assortment of novel antibodies. For example, PCR methods have been used for engineering antibodies with increased affinity to antigen, for "humanizing" antibodies, and for modulating effector function (Marks, J.D., A.D. Griffiths, M.

10 Malmqvist, T. Clackson, J.M. Bye and G. Winter. 1992. Bypassing immunization: high affinity human antibodies by chain shuffling. Bio/Technology 10:779-783; Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96

15 Fab. J. Biol. Chem. 271:22611-22618; Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, Fc γ RI and Fc γ RIII binding. Immunology. 86:319-324).

20 As part of a more comprehensive study, we desired to introduce various site specific mutations in the CH₂ constant domain of human IgG₁. Six specific amino acid residues distributed throughout the CH2 domain previously identified to play a role in immune effector function were marked as targets for mutagenesis (Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-

25 terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, Fc γ RI and Fc γ RIII binding. Immunology. 86:319-324; Duncan, A.R. and G. Winter. 1988. The binding site for Clq on IgG. Nature 332:738-740; Tao, M.-H., R.I.F. Smith and S.L. Morrison. 1993. Structural features of human immunoglobulin G that determine isotype-specific differences in complement

activation. J.Exp.Med. 178:661-667). five of the six residues were grouped into two clusters-one cluster consisting of two residues, two amino acids apart (Location 1, or L1); and a second cluster consisting of three residues spanning a sequence of five amino acids (L2). The remaining amino acid position (L3) made for the total of six 5 residues. We were interested in constructing a panel of mutant CH₂ domain IgGs consisting of each L mutation by itself as well as in combination with other L mutants (e.g., L1; L1; and L2; L1, L2 and L3; etc.).

Various *in vitro* methods have been described where PCR is used to simultaneously 10 introduce distally located site-specific mutations within a gene sequence (Ho, S.N., H.D. Hunt, R.M. Horton, J.K. Pullen and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension. Gene 77:51-59; Ge, L. and P. Rudolph. 1996. Simultaneous introduction of multiple mutations using overlap extention PCR. BioTechniques 22:28-30). Alternatively, an *in vivo* procedure termed recombination 15 PCR (RPCR) has also successfully been used for rapidly and efficiently generating distally located site-specific mutations (Jones, D.H. and S.C. Winistorfer. 1993. Use of polymerase chain reaction for making recombinant constructs. p.241-250. In B.A. White (Ed.), Methods in Molecular Biology, Vol. 15. Humana Press Inc., Totowa, NJ, Jones, D.H. And B.H. Howard. 1991. A rapid method for 20 recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10:62-66). RPCR uses *E. Coli*'s recombination machinery to generate intact circular recombinant plasmids from a transfected mixture of linear PCR-generated product and linearized vector. *In vivo* recombination is mediated through the joining of nucleotide sequences designed into 25 the 5' ends of both PCR primers that are homologous to DNA sequences encoded by the vector. In this report we describe an extension of the RPCR procedure for simultaneously introducing complex combinations of mutations into an antibody CH₂ domain.

Humanized BR96 variable region heavy and light chain genes, previously cloned and co-expressed as an assembled active Fab fragment in an M13 phage expression vector, provided the starting material (Rosok, M.J.. D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D.

5 Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. *J. Biol. Chem.* 271:22611-22618). The heavy and light chain V genes were amplified by PCR from a single-stranded M13 DNA template and subcloned by *in vivo* recombination (Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by

10 placing homologous ends on DNA using polymerase chain reaction. *BioTechniques* 10:62-66) into vectors pD17-hG1a and pD16-hC κ , to form pBR96-hG1a and pBR96-hC κ respectively. pD17-hG1a and pD16-hC κ are eukaryotic immunoglobulin expression vectors derived from pcDNA3 (Invitrogen, San Diego, CA). The plasmid pBR96-hG1a was further modified by site-directed mutagenesis

15 to introduce two Eco47-III restriction sites flanking the immunoglobulin hinge-CH₂-CH₃ domains using standard procedures. The recipient vector was then prepared by digesting pBR96-hG1a with Eco47-III, isolating the vector backbone by agarose gel electrophoresis followed by extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA).

20

The strategy for introducing multiple mutations within the immunoglobulin CH₂ gene, shown in Figure 24, relies on the *in vivo* homologous recombination of several independently amplified PCR products with each other as well as with the pBR96-hG1a vector DNA. For introducing mutations at two distal locations two PCR

25 products are synthesized (Figure 24B). One end of each PCR product is for recombining with an homologous end of the linear vector, and the other end, encoding the mutation(s) of interest, is for recombining with the neighboring PCR product. As shown in Figure 24B, additional distally-located mutations can be introduced into a target sequence by increasing the number of PCR products

proportionately. The recombination of neighboring PCR products always occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends (e.g., A1, A2) contain complementary mutant residues.

The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers (Rs and Ra) contain sequences for recombining with the end regions of the Eco47-III digested pBR96-hG1a vector.

- 10 Each L mutation was amplified in a separate PCR reaction. The reaction conditions were 250 ng intact pBR96-hG1a DNA template, 10 ul of 1X *Pfu* buffer (Stratagene, Inc. San Diego, CA), 10 nmol dNTPs, 200ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase in a 100ul reaction volume. Samples were first denatured at 95° C for 5 min, cooled to 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, followed by a final extension at 72°C for 7 min in a Perkin-Elmer DNA Thermal Cycler (Norwalk, CT). The amplified products were purified from a 1% agarose gel, extracted with Qiagen Gel Extraction kit and the recovered DNA quantitated. 50 ng of each PCR product was mixed with 25 ng of the Eco47-III digested pBR96-hG1a vector, transfected into Max competent E. coli DH5 α according to the manufacturer's procedure (GIBCO BRL/Life Technologies, Gaithersburg, MD), and the entire transfection reaction plated onto selective LB agar plates containing 100 ug/ml ampicillin.

25

The results of several cloning experiments are summarized in the Table that follows. Typically the transformations produced from 80 to 200 bacterial colonies. Individual colonies were selected and grown overnight in 2 ml liquid cultures for isolation of miniprep plasmid DNA (Qiagen) and analysis by Eco47-III restriction

endonuclease mapping. Among 24 independent transformants analyzed from triple homologous recombination events (two PCR products plus vector) 11 clones contained the predicted 1.4 kpb DNA insert.

5 Figure 25 shows a sample diagnostic restriction analysis of DNA prepared from clones derived from quadruple homologous recombination events (three PCR products plus vector). Additional sampling of clones resulting from quadruple recombination yielded a cloning efficiency of 29% (7 clones containing inserts/24 clones sampled). At this point, due to the small sampling sizes, we do not know
10 whether the differences in the cloning efficiencies observed between the triple and quadruple recombination events are meaningful.

To evaluate the expression of Le^y -binding activity of the CH₂ mutant IgGs, miniprep DNAs from 6 clones derived from the triple recombination reaction and 6
15 clones derived from the quadruple recombination reaction exhibiting the predicted diagnostic Eco47-III restriction patterns were isolated, mixed with pBR96- hCκ DNA and used to co-transfect COS7 cells. 48 hour spent supernatants from 3 ml cultures were assayed for total IgG production and for Le^y binding activity by enzyme-linked immunosorbent assay (EIA) as described (Yelton, D.E., M.J. Rosok,
20 G.A. Cruz, W.L. Cosand, J. Bajorath, I. Hellstrom, K.-E. Hellstrom, W.D. Huse and S.M. Glaser. 1995. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. J.Immunol. 155:1994-2004). All twelve cultures were found to secrete approximately 2-3 ug/ml Le^y -reactive IgG. The spectrum of Le^y binding activities were all similar to that of native humanized BR96 IgG indicating
25 that the homologously recombined antibodies did not acquire any gross mutations that could affect antigen binding. To confirm that the desired CH₂ mutations had been incorporated, and to evaluate the recombined genes for misincorporated nucleotides, four of the clones producing functional antibody were sequenced using Sequenase Version 2 DNA Sequencing Kit (United States Biochemical). One clone

was found to contain a single nucleotide change within the forward PCR primer used for mediating recombination with vector DNA. We are uncertain whether this error occurred during chemical synthesis of the oligonucleotide primer or is a result of misincorporation during the PCR reaction, despite the fact that we used a
5 thermostable polymerase with proofreading activity.

A RPCR procedure for homologously recombining up to three separate PCR-generated mutated antibody sequence products into a eukaryotic expression vector for the rapid construction of engineered IgG molecules is described herein. The
10 advantage of this approach is the ability to simultaneously introduce multiple distally-located mutations with PCR products synthesized by a single round of PCR. Recombinant DNAs are produced with a reasonably high cloning efficiency and fidelity of correct nucleotide sequences. The ability to efficiently rejoin several distinct PCR products should permit combinatorial strategies for constructing
15 complexly mutated protein domains as well as broadening the number and location of desired mutations.

Analysis of transformants generated by multiple-fragment RPCR.

Mutant IgGs Constructed	PCR Fragments in reaction	HR ^a events	Colonies Analyzed	Cloning Efficiency ^b
2	2	triple	24	45%
2	3	quadruple	24	33%

^aHR-homologous recombination

^bCloning efficiency (number of clones containing 1.4kbp insert/total number of colonies)

EXAMPLE 5

This example provides two methods for introducing site specific mutations into the
5 CH2 domain of human IgG1 constant region containing vectors.

One method involves PCR amplification of a segment or segments of the constant
region, wherein mutations are introduced using appropriately constructed
oligonucleotides. The vector receiving the fragment(s) is digested with a restriction
10 enzyme to linearize the vector. PCR amplification primers are designed so that the
5' ends of the PCR fragments can hybridize to the DNA sequence of the vectors. If
more than one PCR fragment is amplified, then common sequences to the two
fragments are introduced by oligonucleotides. Bacteria are transfected with the PCR
fragments and with the digested vector. The fragments and vector can recombine by
15 homologous recombination using the bacteria's recombination machinery. Bacterial
colonies are selected and the DNA is analyzed by size and restriction map as a
preliminary determination that the vector and fragment(s) recombined correctly.
Correct insertion of fragments with the mutations is confirmed by dideoxynucleotide
sequence analysis. DNA is then introduced into mammalian cells as described for
20 the CH2 deleted antibody, and the expressed antibody analyzed for binding and
functional activity.

By way of example, mutations Leu to Ala at residue 235 in CH2 and Gly to Ala at
residue 237 were introduced by the procedure disclosed in Example 4. The heavy
25 chain vector used for this procedure was pD17-hG1a, similar to pD17-BR96 vector
described herein except that humanized V regions (Rosok, M.J., D.E. Yelton, L.J.
Harris, J. Bajorath, K-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin,
W.D. Huse, and S.M. Glaser, 1996. J. Biol. Chem 271 37:22611-22618) with three
affinity mutations (H1, H2, and H3 mutations) were substituted.

pBR96-hG1a contains two Eco47-III restriction sites flanking the Ig hinge-CH2-CH3 domains. The recipient vector was prepared by (1) digesting pBR96-hG1a with *Eco*47-III, (2) isolating the vector by agarose gel electrophoresis, and (3) extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA). To introduce mutations at a single location, such as for positions 235 and 237, two PCR products were synthesized.

To introduce two distally located mutations, such as for mutant F (also referred to herein as hBR96-2F) with mutations at 235, 237, 331, requires 3 PCR products. The recombination of neighboring PCR products occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends contain complementary mutant residues. The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers containing sequences for recombining with the end regions of the *Eco*47-III digested pBR96-hG1a vector.

PCR amplification used 250 ng intact pBR96-hG1a DNA template, 10 µl of 10X *Pfu* buffer (Stratagene, Inc., San Diego, CA), 10 nmol dNTPs, 200 ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase (Stratagen, Inc. San Diego, CA) in 100 µl reaction. Samples were denatured at 95°C for 5 min, annealed at 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, and a final extension at 72°C for 7 min. The amplified products were purified from a 1% agarose gel, extracted with the Qiagen Gel Extraction kit and quantitated. 50 mg of each PCR product was mixed with 25 ng of the *Eco*47-III digested pBR96-hG1a vector and transfected in E.coli MAX Efficiency DH5 α ™ according to the

manufacturer's instructions (GIBCO BRL/Life Technologies, Gaithersburg, MD). The entire transfection reaction was plated onto LB agar plated containing 100 µg/ml ampicillin.

- 5 Bacterial colonies were selected and grown overnight at 37° C in 2 ml liquid cultures. DNA was isolated and analyzed by Eco47-III restriction endonuclease mapping. Clones with the correct size insert were sequenced (Sequenase Version 2, U.S. Biochemical Corp., Cleveland, OH).
- 10 The second method for introducing site specific mutations into the CH₂ domain of human IgG1 involved the method of Kunkel (1987 Methods Enzymology, *supra*). For this procedure pD17-hG1b DNA with the F1 origin of replication was introduced into electrocompetent *E. coli* CJ236 dut-ung- (Bio-Rad Laboratories, Hercules, CA) by electroporation according to manufacturer's instructions. PD17-
- 15 hG1b is a vector having a constant region but no variable region. The F1 ori site allows treatment of this vector as a phagemid.

- Bacteria containing the plasmid were selected by ampicillin resistance. Single stranded uridylated DNA was prepared using the Muta-Gene Phagemid In Vitro Mutagenesis Version 2 protocol (Bio-Rad). Mutations were introduced by site-directed mutagenesis with the appropriate antisense oligonucleotide. For molecules with mutations at more than one location, mutations were introduced by either of the two methods discussed above. One method would be to (1) prepare one mutant, for example, mutant 2C (also referred to herein as BR96-2C) with the mutations at residues 318, 320, 322, (2) isolate ssDNA, and (3) introduce a second mutation set with the appropriate anti-sense oligonucleotide. The second method would be to anneal two antisense oligonucleotides with the same uridylated ssDNA and screen for mutants with both sets of changes. Mutant 2H (hBR96-2H) was also prepared by a combination of these methods.

The V region of humanized BR96-2 heavy chain was introduced by the homologous recombination method described above in pD17-hJm14.H1. The pD17-hJm14.H1 plasmid contains the BR96 humanized variable region with the H1/H2/H3 mutations and the plasmid was used to transfect mutant sequences into mammalian cells. The pD17G1b vector containing the Fc mutation(s) was digested with NheI for 3 hr at 37° C and the DNA isolated by methods described above. Insertion of the V region into the vector was determined by size and restriction enzyme mapping and confirmed by sequence analysis.

10

Transient expression of whole antibodies was performed by transfection of COS cells. For production of antibody, stable transfections of CHO cells were performed (see description of deleted CH2 mutant). All mutants were purified from CHO culture supernatants by protein A chromatography.

15

The oligonucleotide primers homologous to the vector and used to introduce the constant regions mutations were as follows:

Oligonucleotides homologous to vector sequences:

Sens(sense)CH2 E47-3-5: CAG GGA GGG AGG GTG TCT GCT GGA AGC

20 CAG GCT CAG CGC TGA CCT CAGA

D CH2 E47-3 A (antisense): GGA AAG AAC CAT CAC AGT CTC GCA GGG
GCC CAG GGC AGC GCT GGG TGC TT

Oligonucleotides to mutate Leu235 to Ala and Gly237 to Ala (underlined sequences show sites of mutation):

Antisense CH2 L235-G237/aa: GAA GAG GAA GAC TGA CGG TGC CCC
CGC GAG TTC AGG TGC TGA GG

SensCH2 L235-G237/AA: CCT CAG CAC CTG AAC TCG CGG GGG CAC
CGT CAG TCT TCC TCT TC

Oligonucleotides to mutate Glu318, Lys320, Lys322 to Ser

Antis(antisense)CH2 EKK/SSS-2: CTG GGA GGG CTT TGT TGG AGA CCG
AGC ACG AGT ACG ACT TGC CAT TCA GCC

5 Oligonucleotides to mutate Pro331 to Ala:

Antis CH2 P331/A/3: GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC

Sense CH2 P33/A: GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC

Alternative antisense oligo to introduce Ala at 331 by site-directed mutation:

CH2P331A: GAT GGT TTT CTC GAT AGC GGC TGG GAG GGC TTT G

10

Oligonucleotides to mutate Glu318 to Ser, Lys320 to Ser, Lys322 to Ser, and Pro331 to Ala:

Antis CH2 EKKP/SSA-6: GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC TTT GTT GGA GAC CGA GCA CGA GTA CGA CTT GCC ATT CAG

15 CCA GTC CTG GTG

Sense CH2 EKKP/SSA-6: CAC CAG GAC TGG CTG AAT GGC AAG TCG TAC TCG TGC TCG GTC TCC AAC AAA GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC

20

In vitro Assays of the Mutants

Results of the CDC demonstrate that mutant hBR96-2B has approximately 10 fold less activity than the control hBR96-1 (two affinity mutations, one in H2 and one in 25 H3, refer to previous patent (Figure 20)). The mutants that have the least ability to kill cells in the presence of complement is hBR96-2C with the triple mutations at positions 318, 320, and 322 and the hBR96-2H mutant (least cytotoxic antibodies in the panel) which contains all six mutations at the three different locations. ADCC activity was most affected by the CH2 deleted hBR96-2 molecule (Figure 21).

hBR96-2B and -2H lost between 100 and 1000 fold activity to kill in the presence of effector cells. In the ADCC assay the hBR96-2B molecule also lost approximately 10 fold activity (Figure 21).

- 5 Figures 26-28 provide the amino acid sequences for the heavy chain variable region for both chimeric and humanized BR96 having the H1, H2, and H3 mutations. The amino acid sequence for the light chain variable region is known and methods for generating it are found in PCT Application No. 95/305444. Additionally provided is the amino acid sequence for the IgG1 constant region. Mutations in the constant
10 region are marked.

SEQUENCE LISTING

(1) GENERAL INFORMATION

5

(i) APPLICANT: Bristol-Myers Squibb Co.

(ii) TITLE OF THE INVENTION:

10

A METHOD FOR INHIBITING
IMMUNOGLOBULIN-INDUCED TOXICITY FROM THE USE OF
IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS

(iii) NUMBER OF SEQUENCES: 13

15

(iv) CORRESPONDENCE ADDRESS:

20

- (A) ADDRESSEE: Merchant & Gould
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- (E) COUNTRY: USA
- (F) ZIP: 90025

25

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Diskette
- (B) COMPUTER: IBM Compatible
- (C) OPERATING SYSTEM: DOS
- (D) SOFTWARE: FastSEQ Version 2.0

30

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER: PCT/US97/_____.
- (B) FILING DATE: 01-AUG-1997
- (C) CLASSIFICATION:

35

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER: 60/023,033
- (B) FILING DATE: 02-AUG-1996

40

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50

(2) INFORMATION FOR SEQ ID NO:1:

55

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 TGGCACCGAA AGCTTTCTGG GGCAGGCCAG GCCTGA

36

(2) INFORMATION FOR SEQ ID NO:2:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 57 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

20 TCCGGACATG TTGGTACCCA CGTGGTGGTC GACGCTGAGC CTGGCTTCGA GCAGACA

57

(2) INFORMATION FOR SEQ ID NO:3:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 55 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCGACCACC ACCTGGGTAC CAACATGTCC GGAGCCACAT GGACAGAGGC CGGCT

55

35 (2) INFORMATION FOR SEQ ID NO:4:

40 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTGGTTCTTG TTCATCTCCT CTCTAGATGG

30

50 (2) INFORMATION FOR SEQ ID NO:5:

55 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACCATGGTCG ACCTCAGACC TGCCAAGAGC CATATC

36

5 (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATGGTCACG TGGTGTGTCC CTGGATGCAG GCTACTCTA

39

20 (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

30 CAGGGAGGGA GGGTGTCTGC TGGAAGCCAG GCTCAGCGCT GACCTCAGA

49

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

45 GGAAAGAACCG ATCACAGTCT CGCAGGGGCC CAGGGCAGCG CTGGGTGCTT

50

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8691 base pairs
50 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

60 GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA GCCAGAGTAA
CCTTTTTTTT TAATTTTATT TTATTTTATT TTTGAGATGG AGTTGGCGC CGATCTCCCG

120

	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTCGCTG	AGTAGTGCAC	GAGCAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCAC	300
5	TGCTTCGCGA	TGTACGGGCC	AGATATAACGC	GTGACATTG	ATTATTGACT	AGTTATTAAT	360
	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCCATATAT	GGAGTTCGC	GTTACATAAC	420
	TTACCGTAAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATG	ACCTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTACCGTA	AACTGCCAC	TTGGCAGTAC	ATCAAGTGT	TCATATGCCA	AGTACGCC	600
10	CTATTGACGT	CAATGACCGT	AAATGGCCCG	CCTGGCATT	TGCCCCGAC	ATGACCTTAT	660
	GGGACTTCCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTG	CTCACGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACC	AAATCAACGG	GACTTCCAA	840
	AATGCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCG	TAGGCGTGT	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
15	TTAACACGAC	TCACTATAGG	GAGACCAAG	CTTGGTACCA	ATTAAATTG	ATATCTCCTT	1020
	AGGCTCTGAG	TCTCTAGATA	ACCGGTCAT	CGATTGGAAT	TCTTGGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCC	TGTTTAA	GGTGTCCAGT	1140
	GTGAAGTGA	TCTGGTGGAG	TCTGGGGAG	GCTTAGTGC	GCCTGGAGGG	TCCCTGAAAG	1200
20	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTCGCCAGA	1260
	CTCCAGAGAA	GAGGCTGGAG	TGGGTCGCAT	ACATTAGTC	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTCACCA	TCTCCAGAGA	CAATGCCAAG	AAACCCCTGT	1380
	ACCTGCAAAT	GAGCCGCTG	AACTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
25	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTGCGT	1620
	GGAACTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCCTA	CAGTCCTCAG	1680
	GAECTACTC	CCTCAGCAGC	GTGGTCACCG	TGCCCTCCAG	CAGCTTGGC	ACCCAGACCT	1740
30	ACATCTGAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTGGTGAGA	1800
	GGCCAGCACA	GGGAGGGAGG	GTGTCGCTG	GAAGCCAGGC	TCAGCCTC	TGCCCTGGACG	1860
	CATCCCAGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGCTC	CCTCTTCACC	1920
	CGGAGGCCCTC	TGCCCCGCCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTCTCCAG	1980
	GCTCTGGGCA	GGCACAGGCT	AGGTCCCCCT	AACCCAGGCC	CTGCACACAA	AGGGCAGGT	2040
35	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCCCTGA	CCTAAGCCCA	2100
	CCCCAAAGGC	CAAACCTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
	GTAACCTCCA	ATCTTCTCTC	TGAGAGGCC	AAATCTTGT	ACAAAACCTA	CACATGCCA	2220
	CCGTGCCCCAG	GTAAGCCAGC	CCAGGCCCTC	CCCTCCAGCT	CAAGGGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACAGGGCCCA	GCCGGGGTGT	GACACGTCA	CCTCCATCTC	2340
	TTCTCAGCA	CCTGAACCTC	TGGGGGGACC	GTCAGTCTTC	CTCTTCCCCC	CAAACCCAA	2400
	GGACACCCCTC	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	GTGGTGGTGG	ACGTGAGCCA	2460
40	CGAACACCC	GAGGTCAAGT	TCAACTGGT	CGTGGACGGC	GTGGAGGTGC	ATAATGCCAA	2520
	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG	CACGTACCGT	GTGGTCAGCG	TCCTCACCGT	2580
	CCTGCACCAAG	GAETGGCTGA	ATGGCAAGGA	GTACAAGTGC	AAGGTCTCCA	ACAAAGCCCT	2640
	CCCAGCCCC	ATCGAGAAAA	CCATCTCAA	AGCCAAAGGT	GGGACCCGTG	GGGTGCGAGG	2700
	GCCACATGGA	CAGAGGCCGG	CTCGGCCAC	CCTCTGCCCT	GAGAGTGC	GCTGTACCAA	2760
45	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGA	CACCTGCC	CCATCCCGGG	2820
	ATGAGCTGAC	CAAGAACAC	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCCAGCG	2880
	ACATGCCGT	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCTC	2940
	CCGTGCTGGA	CTCGACGGC	TCCTTCTTCC	TCTACAGCA	GCTCACCGT	GACAAGAGCA	3000
	GGTGGCAGCA	GGGAAACGTC	TTCTCATGCT	CCGTGATGC	TGAGGCTCTG	CACAACCACT	3060
50	ACACGCAGAA	GAGCCTCTCC	CTGTCTCCGG	GTAAATGAGT	GCGACGGCCG	GCAAGCCCCC	3120
	GCTCCCCGGG	CTCTCGCGT	CGCACCGAGG	TGCTTGGCAC	GTACCCCTG	TACATACTTC	3180
	CCGGCGGCC	AGCATGGAAA	TAAAGCACCC	AGCGCTGCC	TGGGCCCTG	CGAGACTGTG	3240
	ATGGTTCTTT	CCACGGGTC	GGCCGAGTCT	GAGGGCTGAG	TGGCATGAGG	GAGGCAGAGC	3300
	GGGTCCCCACT	GTCCCCCAC	TGGCCAGGC	TGTGAGGTG	TGCCTGGGCC	CCCTAGGGTG	3360
55	GGGCTCAGCC	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATT	GCCAGCGTGG	CCCTCCCTCC	3420
	AGCAGCACCT	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTGGG	GACAGACACA	3480
	CAGCCCCCTG	CTCTGTAGGA	GACTGCTCTG	TTCTGTGAC	GCCCCCTGTC	TCCCGACCTC	3540
	CATGCCCACT	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGGCCCTC	CCTCACCCAT	3600
	CTACCCCCAC	GGCACTAAC	CTTGGCTGCC	CTGCCAGGCC	TCGCACCCGC	ATGGGGACAC	3660

	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTCA	GATGCCACA	3720
	CACACACTCA	GCCCAGACCC	GTTAACAAA	CCCCGACTG	AGTTGGCCG	GCCACACGGC	3780
	CACCAACAC	ACACGTGAC	GCCTCACACA	CGGAGCCTA	CCCGGGCAGA	CTGCACAGCA	3840
	CCCAGACCAG	AGCAAGGTCC	TCGCACACGT	GAACACTCCT	CGGACACAGG	CCCCCACGAG	3900
5	CCCCACGCGG	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	3960
	TCAGACAAAC	CCAGCCCTCC	TCTCACAAAGG	GTGCCCCCTGC	AGCCGCCACA	CACACACAGG	4020
	GGATCACACA	CCACGTCAG	TCCCTGGCCC	TGGCCCACTT	CCCACTGCGC	CCCTTCCCTG	4080
	CAGGACGGAT	CAGCCTCGAC	TGTGCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCCCTCC	4140
	CCCGTGCCTT	CCTTGACCCCT	GGAAAGGTGCC	ACTCCCCTGT	TCCTTCCCTA	ATAAAATGAG	4200
10	GAAATTGCAT	CGCATTGTCT	GAGTAGGTGT	CATTCTATT	TGGGGGGTGG	GGTGGGGCAG	4260
	GACAGCAAGG	GGGAGGATG	GGAAAGACAAT	AGCAGGCCAT	CTGGGGATGC	GGTGGGGCTCT	4320
	ATGGCTCTG	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	4380
	AGCGGCGCAT	TAAGCGGGC	GGGTGTTGTG	TTACGCGCA	CGTGACCGC	TACACTTGCC	4440
	AGCGCCCTAG	CGCCCCCTCC	TTTCGCTTTC	TTCCCTCCCT	TTCTCGCCAC	GTCGCCGGG	4500
15	CCTCTAAAG	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCAGCC	4560
	CTAACTCCGC	CCATCCCCC	CCTAACTCCG	CCCAGTTCCG	CCCATTCTCC	GCCCCATGGC	4620
	TGACTAATTT	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCC	CGGCCTCTGA	GCTATTCCAG	4680
	AAGTAGTGTG	GAGGCTTTTT	TGGAGGCCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	4740
	GCTGCGATT	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	4800
20	CCCGCTGCCA	TCATGGTTCG	ACCATTGAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	4860
	ATTGGCAAGA	ACGGAGACCT	ACCCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	4920
	AGAATGACCA	CAACCTCTTC	AGTGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	4980
	ACCTGGTTCT	CCATTCCCTGA	GAAGAATCGA	CCTTAAAGG	ACAGAATTAA	TATAGTTCTC	5040
	AGTAGAGAAC	TCAAAGAAC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTGGATGAT	5100
25	GCCTTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	5160
	GGAGGCAGTT	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	5220
	ACAAGGATCA	TGCAAGGATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	5280
	TATAAACTTC	TCCCAGAATA	CCCAGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	5340
	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA	GAATACAGG	AAGATGCTTT	CAAGTTCTCT	5400
30	GCTCCCTCC	TAAAGCTATG	CATTTTATA	AGACCATGGG	ACTTTGCTG	GCTTTAGATC	5460
	TCTTTGTGAA	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAAACTA	CCTACAGAGA	5520
	TTTAAAGCTC	TAAGCTTAAAT	ATAAAATTTT	TAAGTGTATA	ATGTGTTAAA	CTACTGATTC	5580
	TAATTGTTTG	TGTATTTTAG	ATTCCAACCT	ATGGAACCTGA	TGAATGGGAG	CAGTGGTGG	5640
	ATGCCTTAA	TGAGGAAAAC	CTGTTTGTCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	5700
35	CTACTGCTGA	CTCTCAACAT	TCTACTCCCTC	CAAAAGAGAA	GAGAAAGGTA	GAAGACCCCA	5760
	AGGACTTTCC	TTCAGAAATTG	CTAAAGTTTG	TGAGTCATGC	TGTGTTAGT	AAAGAAACTC	5820
	TTGCTTGCTT	TGCTATTTAC	ACCACAAAGG	AAAAGCTGC	ACTGCTATAC	AAAGAAAATTA	5880
	TGGAAAATTA	TTCTGTAAAC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	5940
	TTTTCTTAC	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTGT	6000
40	GTACCTTTAG	CTTTTTAATT	TGTAAGGGG	TTAATAAGGA	ATATTGATG	TATAGTGCCT	6060
	TGACTAGAGA	TCATAATCAG	CCATACCA	TTTGTAGAGG	TTTACTTGC	TTTAAAAAAAC	6120
	CTCCCACACC	CCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	6180
	TTTATTGCGA	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATT	CACAAATAAA	6240
	GCATTTTTT	CACTGCATTC	TAGTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	6300
45	GTCTGGATCG	GCTGGATGAT	CCTCCAGCGC	GGGGATCTA	TGCTGGAGTT	CTTCGCCAC	6360
	CCCAACTTGT	TTATTGCGAC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTC	6420
	ACAAATAAAAG	CATTTTTTTC	ACTGCATTCT	AGTTGTGGT	TGTCCAAACT	CATCAATGTA	6480
	TCTTATCATG	TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	6540
	CTGTTCCCTG	TGTGAAATTG	TTATCCGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	6600
50	ATAAAGTGT	AAGCCTGGGG	TGCTTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	6660
	TCACTGCCCC	CTTTCCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCTTAATG	AATCGGCCAA	6720
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	CTGGCGCTCG	TCGTTCGCT	GGGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	6840
	TTATCCACAG	AATCAGGGGA	TAACCGAGGA	AAGAACATGT	GAGCAAAGG	CCAGCAAAG	6900
55	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTCC	ATAGGCTCCG	CCCCCTGAC	6960
	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	7020
	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCGCTT	7080
	ACCGGATACC	TGTCCGCCTT	TCTCCCTTCG	GGAAAGCGTGG	CGCTTCTCA	ATGCTCACGC	7140
	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	7200

	CCCGTTCA	CCGACCGCTG	CGCCTTATCC	GGTAACATAC	GTCTTGAGTC	CAACCCGGTA	7260
	AGACACGACT	TATGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	7320
	GTAGGCCTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTA	ACGGCTACAC	TAGAAGGACA	7380
5	GTATTTGGTA	TCTCGCTCT	GCTGAAGCCA	GTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	7440
	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	7500
	ACGCGCAGAA	AAAAGGATC	TCAAGAAGAT	CCTTGATCT	TTTCTACGGG	GTCTGACGCT	7560
	CAGTGGAACG	AAAACACG	TTAAGGGATT	TTGGTCATGA	GATTATCAA	AAGGATCTTC	7620
	ACCTAGATCC	TTTTAAATT	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	7680
10	ACCTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	7740
	TTTCGTTCAT	CCATAGTTGC	CTGACTCCCC	GTGCGTGTAGA	TAACTACGAT	ACGGGAGGGC	7800
	TTACATCTG	CCCCCAGTGC	TGCAATGATA	CCCGCAGAC	CACGCTCAC	GGCTCCAGAT	7860
	TTATCAGCAA	AAAACCAGCC	AGCCGGAAGG	CCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	7920
	TCCGCTCCA	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCGCCAGTT	7980
15	AATAGTTGC	GAACAGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACG	CTCGTCGTTT	8040
	GGTATGGCTT	CATTCA	CGGTTCCC	CGATCAAGG	GAGTTACATG	ATCCCCCATG	8100
	TTGTGCAAAA	AAGCGGTTAG	CTCCTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTTGGCC	8160
	GCAGTGTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	8220
	GTAAGATGCT	TTTCTGTGAC	TGGTGAAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	8280
20	CGGGCACC	GTTGCTCTG	CCCGCGTCA	ATACGGATA	ATACCGGCC	ACATAGCAGA	8340
	ACTTAAAAG	TGCTCATCAT	TGGAAAACGT	TCTTCGGGC	GAAAACCTC	AAGGATCTTA	8400
	CCGCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAAC	GTAC	8460
	TTTACTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	8520
	GGAATAAGGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTCA	ATATTATTGA	8580
25	AGCATTTATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	8640
	AAACAAATAG	GGGTTCCCG	CACATTCCC	CGAAAAGTGC	CACCTGACGT	C	8691

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

30	(A) LENGTH: 8327 base pairs
	(B) TYPE: nucleic acid
	(C) STRANDEDNESS: single
	(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGCGCCG	GCTTCGAATA	GCCAGAGTAA	60
40	CCTTTTTTTT	TAATTTTATT	TTATTTTATT	TTTGAGATGG	AGTTTGGCGC	CGATCTCCG	120
	ATCCCTATG	GTGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTTGTG	GAGGTGCGCTG	AGTAGTGC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGC	300
	TGCTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAA	360
45	AGTAATCAAT	TACGGGGTCA	TTAGTTCAT	GCCCATA	GGAGTTC	CGTACATAAC	420
	TTACGGTAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTACGGTA	AACTGCCAC	TTGGCAGTAC	ATCAAGTGT	TCATATGCCA	AGTACGCC	600
50	CTATTGACGT	CAATGACGGT	AAATGGCCCG	CCTGGCATT	TGCCCAGTAC	ATGACCTTAT	660
	GGGACTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATT	ACATGGTATG	720
	GGTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTG	CTCACGGG	TTTCCAAGTC	780
	TCCACCCAT	TGACGTCAAT	GGGAGTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCAA	840
	AATGCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGCGG	TAGGC	GTGTA CGGTGGGAGG	900
55	TCTATATAAG	CAGAGCTCTC	TGGCTAA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
	TTAATACGAC	TCACTATAGG	GAGACCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTC	1020
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	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCT	TGTTTAAAAA	GGTGTCCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATT	CATGTATTG	GTTCGCCAGA	1260

	CTCCAGAGAA GAGGCTGGAG TGGGTGCGAT ACATTAGTCA AGGTGGTGAT ATAACCGACT	1320
	ATCCAGACAC TGTAAGGGT CGATTACCCA TCTCCAGAGA CAATGCCAAG AACACCCCTGT	1380
5	ACCTGCAAAT GAGCCGCTCG AAGTCTGAGG ACACAGCCAT GTATTACTGT GCAAGAGGCC	1440
	TGGACGACGG GGCCCTGGTT GCTTACTGGG GCCAAGGGAC TCTGGTCACG GTCTCTGTAG	1500
	CTAGCACCAA GGGCCCATCG GTCTTCCCCC TGGCACCCCTC CTCCAAGAGC ACCCTCTGGGG	1560
	GCACAGCGGC CCTGGCTGCTC CTGGTCAAAGG ACTACTTCCC CGAACCGGTG ACGGTGTCGT	1620
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10	ACATCTGCAA CGTGAATCAC AAGCCAGCA ACACCAAGGT GGACAAGAAA GTTGGTGAGA	1800
	GGCCAGCACAA GGGAGGGAGG GTGTCTGCTG GAAGCCAGGC TCAGCGCTCC TGCTGGACG	1860
	CATCCCGGCT ATGCAGCCCC AGTCCAGGGC AGCAAGGCAG GCCCCGCTCG CCTCTTCACC	1920
	CGGAGGCCTC TGCCCGCCCC ACTCATGCTC AGGGAGGG TCTTCTGGCT TTTTCCCCAG	1980
	GCTCTGGGCA GGCACAGGCT AGGTGCCCCT AACCAGGCC CTGACACAA AGGGGCAGGT	2040
	GCTGGGCTCA GACCTGCCAA GAGCCATATC CGGGAGGACC CTGCCCCCTGA CCTAAGGCCA	2100
15	CCCCAAAGGC CAAACTCTCC ACTCCCTCAG CTCGGACACC TTCTCTCTC CCAGATTCCA	2160
	GTAACCTCCA ATCTTCTCTC TGCAGAGCCC AAATCTTGTG ACAAAACCTCA CACATGCCA	2220
	CCGTGCCCCAG GTAAGCCAGC CCAGGCTCG CCCTCCAGCT CAAGGGGGG CAGGTGCCCT	2280
	AGAGTAGCCT GCATCCAGGG ACACACCACG TGGGTACCAA CATGTCCGGA GCCACATGGA	2340
	CAGAGGCCGG CTCGGCCCCAC CCTCTGCCCT GAGAGTGACC GCTGTACCAA CCTCTGTCCC	2400
20	TACAGGGCAG CCCCAGAAC CACAGGTGTA CACCCCTGCC CCATCCCGGG ATGAGCTGAC	2460
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	CTCCGACGGC TCCTTCTTCC TCTACAGCAA GCTCACCGTG GACAAGAGCA GGTGGCAGCA	2640
	GGGAAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT ACACGCAGAA	2700
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35	GGGGACATGC ACTCTGGGC CCTGTGGAGG GACTGGTGCA GATGCCACAA CACACACTCA	3360
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	CACCTCAAGG CCCACGAGCC TCTCGGCAGC TTCTCCACAT GCTGACCTGC TCAGACAAAC	3600
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45	GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT ATGGCTTCTG	3960
	AGGGGAAAG AACCAAGCTGG GGCTCTAGGG GGTATCCCCA CGGCCCTGT AGCGGCCAT	4020
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	CGCCCGCTCC TTTCGCTTTC TTCCCTTCCCT TTCTCGCCAC GTTCGCCGGG CCTCTCAAAA	4140
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50	CCATCCCGCC CCTAACTCCG CCCAGTCCG CCCATTCTCC GCCCCATGGC TGACTAATT	4260
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	GAGGCTTTTT TGGAGGCCTA GGCTTTGCA AAAAGCTTG ACAGCTCAGG GCTGCGATT	4380
	CGCGCCAAAC TTGACGGCAA TCCTAGCGTG AAGGCTGGTA GGATTCTATC CCCGCTGCCA	4440
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	TCAAAGAAC ACCACGAGGA GCTCATTTTC TTGCCAAAAG TTTGGATGAT GCCTTAAGAC	4740
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	CTGTTTACCA GGAAGCCATG AATCAACCAG GCCACCTTAG ACTCTTG TG ACAAGGATCA	4860
	TGCAGGAATT TGAAAGTGC ACGTTTTC CAGAAATTGA TTTGGGAAA TATAAACTTC	4920
	TCCCAGAATA CCCAGCGTC CTCTCTGAGG TCCAGGAGGA AAAAGGCATC AAGTATAAGT	4980
5	TTGAAGTCTA CGAGAAGAAA GACTAACAGG AAGATGCTT CAAGTTCTCT GCTCCCTCC	5040
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	TAAGGTAAT ATAAAATT TT TAAGTGTATA ATGTGTTAAA CTACTGATTC TAATTGTTTG	5220
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10	TGAGGAAAAC CTGTTTGCT CAGAAGAAAT GCCATCTAGT GATGATGAGG CTACTGCTGA	5340
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	TTCTGTAACC TTTATAAGTA GGCATAACAG TTATAATCAT AACATACTGT TTTTCTTAC	5580
15	TCCACACAGG CATAGAGTGT CTGCTATTAA TAACTATGCT CAAAAATTGT GTACCTTCTAG	5640
	CTTTTAAAT TGAAAGGGG TTAATAAGGA ATATTGATC TATAGTGCT TGACTAGAGA	5700
	TCATAATCAG CCATACACCA TTTGTAGAGG TTTTACTTGC TTTAAAAAAC CTCCCACACC	5760
	TCCCCCTGAA CCTGAAACAT AAAATGAATG CAATTGTTGT TGTAACTTG TTTATTGAG	5820
	CTTATAATGG TTACAATAA AGCAATAGCA TCACAAATT CACAAATAAA GCATTTTTT	5880
20	CACTGCATTC TAGTTGTTG TGTCACAAAC TCATCAATGT ATCTTATCAT GTCTGGATCG	5940
	GCTGGATGAT CCTCCAGCGC GGGGATCTCA TGCTGGAGTT CTTCGCCAC CCCAACCTGT	6000
	TTATTGAGC TTATAATGGT TACAATAAA GCAATAGCAT CACAAATTTC ACAAAATAAG	6060
	CATTTTTTC ACTGCATTCT AGTTGTTG TGTCACAACT CATCAATGTA TCTTATCATG	6120
	TCTGTATACC GTCGACCTCT AGCTAGAGCT TGGCGTAATC ATGGTCATAG CTGTTCTG	6180
25	TGTGAAATTG TTATCCGCTC ACAATCCAC ACAACATACG AGCCGAAAGC ATAAAGTGT	6240
	AAGCCTGGGG TGCCTAATGA GTGAGCTAAC TCACATTAAT TGCGTTGCGC TCACTGCCCC	6300
	CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TGCTTAATG AATCGGCCAA CGCGCGGGGA	6360
	GAGCGGTTT CGCTATTGGG CGCTCTTCCG CTTCCCTCGCT CACTGACTCG CTGCGCTCGG	6420
	TCGTCGGCT CGGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAAATACGG TTATCCACAG	6480
30	AATCAGGGGA TAACCGAGGA AAGAACATGT GAGCAAAGG CCAGCAAAGG GCCAGGAACC	6540
	GTAAAAAGGC CGCGTTGCTG GCGTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA	6600
	AAAATCGACG CTCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATAAAGA TACCAAGCGT	6660
	TTCCCCCTGG AAGCTCCCTC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT ACCGGATAACC	6720
	TGTCGCGCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA ATGCTCACGC TGTAGGTATC	6780
35	TCAGTCGGT GTAGGTCGTT CGCTCCAAGC TGGCTGTGTG GCACGAACCC CCCGTTCA	6840
	CCGACCGCTG CGCCTTATTC GGTAACATTC GTCTTGAGTC CAACCCGGTA AGACACGACT	6900
	TATCGCCACT GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCGAGGTAT GTAGGCGGTG	6960
	CTACAGAGTT CTTGAAGTGG TGGCTTAACG ACGGCTACAC TAGAAGGACA GTATTGGTA	7020
	TCTGCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA	7080
40	AACAAACCAC CGCTGGTAGC GGTGGTTTT TTGTTGCAA GCAGCAGATT ACCGGCAGAA	7140
	AAAAAGGATC TCAAGAAGAT CTTTGATCT TTTCTACGG GTCTGACGCT CAGTGGAAACG	7200
	AAAACTCACG TTAAGGGATT TTGGTCATGA GATTATCAA AAGGATCTTC ACCTAGATCC	7260
	TTTAAATTAA AAAATGAAGT TTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG	7320
	ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGCTA TTTCGTTCAT	7380
45	CCATAGTTGC CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG	7440
	GCCCCAGTGC TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA	7500
	TAAACCAGCC AGCCGGAAGG GCGGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA	7560
	TCCAGTCTAT TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTGC	7620
	GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTCAAG CTCGTCGTTT GGTATGGCTT	7680
50	CATTCACTC CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA	7740
	AAGCGGTTAG CTCCCTCGGT CCTCCGATCG TTGTCAGAAG TAAGTGGCC GCAGTGTAT	7800
	CACTCATGGT TATGGCAGCA CTGCATAATT CTCTTACTGT CATGCCATCC GTAAGATGCT	7860
	TTTCTGTGAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG CGCGGACCGA	7920
	GTTGCTCTG CCCGGCGTCA ATACCGGATA ATACCGCGCC ACATAGCAGA ACTTTAAAAG	7980
55	TGCTCATCAT TGGAAAACGT TCTTCGGGGC GAAAACCTCTC AAGGATCTTA CCGCTGTTGA	8040
	GATCCAGTTC GATGTAACCC ACTCGTGCAC CCAACTGATC TTCAGCATCT TTTACTTTCA	8100
	CCAGCGTTTC TGGGTGAGCA AAAACAGGAA GGCAAAATGC CGCAAAAGGG GGAATAAGGG	8160
	CGACACGGAA ATGTTGAATA CTCATACTCT ICCTTTTCA ATATTATTGA AGCATTATTC	8220
	AGGGTTATTG TCTCATGAGC GGATACATAT TTGAATGTAT TTGAAAAAT AACAAATAG	8280
	GGGTTCCCGCG CACATTCCC CGAAAAGTGC CACCTGACGT CCBRAAG	8327

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 8897 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

15	GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGCAC CATGAAGTTG CCTGTTAGGC TGTTGGTGCT GATGTTCTGG ATTCCCTGCTT CCAGCAGTGA TGTTTGATG ACCCAAATTC CAGTCTCCCT GCCTGTAGT CTTGGAGATC AACGCTCCAT CTCTTGAGA TCTAGTCAGA TCATTTGACA TAATAATGGC AACACCTATT TAGAATGGTA CCTGCAGAAA CCAGGCCAGT CTCCACAGCT CCTGATCTAC AAAGTTTCCA ACCGATTTTC TGGGGTCCC GACAGGTTCA GCGGCAGTGG ATCAGGGACA GATTTCACAC TCAAGATCAG CAGAGTGGAG GCTGAGGATC	60 120 180 240 300 360
20	TGGGAGTTA TTACTGCTT CAAGGTTAAC ATGTTCCATT CACGTTGGC TCGGGGACAA AGTTGAAAT AAAACGTAAG TCTCGAGTCT CTAGATAACC GGTCAATCGA TTGGAATTCT AAACTCTGAG GGGGTCGGAT GACGTGGCCA TTCTTGCCT AAAGCATTGA GTTTACTGCA AGGTAGAAA AGCATGAAA GCCCTCAGAA TGGCTGCAA GAGCTCCAAC AAAACAATT AGAACCTTAT TAAGGAATAG GGGGAAGCTA GGAAGAAACT CAAAACATCA AGATTTTAAA	420 480 540 600 660
25	TACGCTCTT GGTCTCCTT CTATAATTAT CTGGGATAAG CATGCTGTT TCTGTCTGTC CCTAACATGC CCTTATCCGC AAACACACCA CCCAAGGGCA GAACTTTGTT ACTTAAACAC CATCCTGTT GCTTCTTCC TCAGGAACCTG TGGCTGCACC ATCTGTCTTC ATCTTCCGC CATCTGATGA GCAGTTGAAA TCTGGAACCTG CCTCTGTTGT GTGCCTGCTG AATAACTTCT ATCCCCAGAGA GGCCAAAGTA CAGTGGAAAGG TGGATAACGC CCTCCAATCG GGTAACTCCC	720 780 840 900 960
30	AGGAGAGTGT CACAGAGCAG GAGAGCAAGG ACAGCACCTA CAGCCTCAGC AGCACCCCTGA CGCTGAGCAA AGCAGACTAC GAGAACACAA AAGTCTACGC CTGCGAAGTC ACCCATCAGG GCCTGAGCTC GCCCGTCACA AAGAGCTTCA ACAGGGGAGA GTGTTAGAGG GAGAAGTGCC CCCACCTGCT CCTCAGTCTTCC AGCCTGACCC CCTCCCCATCC TTTGGCCTCT GACCCTTTTT CCACAGGGGA CCTACCCCTA TTGCGGTCTT CCAGCTCATC TTTCACCTCA CCCCCCTCCT	1020 1080 1140 1200 1260
35	CCTCCTTGGC TTTAATTATG CTAATGTTGG AGGAGAATGA ATAAATAAAG TGAATCTTGT CACCTGTGGT TTCTCTCTT CCTCATTAA TAATTATTAT CTGTTGTTT ACCAACTACT CAATTCTCT TATAAGGGAC TAAATATGT GTCATCTAA GGACAGCTAAC CATTATAAAA AATCATCTT CATTCTATTAC TACCCATCA TCCCTCTGCAA GACAGCTCATC CCTCAAACCC ACAAGCCTC TGTCCTCACA GTCCCCCTGGG CCATGGTAGG AGAGACTTGC TTCCCTGTT	1320 1380 1440 1500 1560
40	TCCCCCTCTC AGCAAGCCCT CATACTCCTT TTTAAGGGTG ACAGGCTTA CAGTCATATA TCCTTGATT CAATTCCCTG AGAATCAACC AAAGCAAATT TTTCAAAGA AGAAACCTGC TATAAAGAGA ATCATTCTT GCAACATGAT ATAAAATAAC AACACAATAA AAGCAATTAA ATAAACAAAC AATAGGGAAA TGTTTAAGTT CATCATGGTA CTTAGACTTA ATGGAATGTC ATGCCTTATT TACATTTTA AACAGGTACT GAGGGACTCC TGTCTGCCAA GGGCGTATT	1620 1680 1740 1800 1860
45	GAGTACTTTC CACAACCTAA TTTAATCCAC ACTATACTGT GAGATTAAGG ACATTCTTAA AAATGTTGCA AAGGTTCTAT AAAGCTGAGA GACAAATATA TTCTATAACT CAGCAATCCC ACTTCTAGAT GACTGAGTGT CCCCCACCCAC CAAAAAACTA TGCAAGAATG TTCAAAGCAG CTTTATTTAC AAAAGCCAAA AATTGGAAAT AGCCCGATTG TCCAACAATA GAATGAGTTA TTAAACTGTG GTATGTTTAC ATATTAGAAT ACCCAATGAG GAGAATTAAC AAGCTACAAC	1920 1980 2040 2100 2160
50	TATACCTACT CACACAGATG AATCTCTAA AAATAATGT ACATAAGAGA AACTCAATGC AAAAGATATG TTCTGTATGT TTTCATCCAT ATAAAGTTCA AAACCGAGTA AAAATAAAGT TAGAAAATTG GATGGAAATT ACTCTTAGCT GGGGGTGGGC GAGTTAGTGC CTGGGAGAAG ACAAGAAGGG GCTTCTGGGG TCTTGGTAAT GTTCTGTTCC TCGTGTGGGG TTGTGCAGTT ATGATCTGTG CACTGTTCTG TATACACATT ATGTTCAAA ATAACCTTCAC ATAAAGAACAA	2220 2280 2340 2400 2460
55	TCTTATACCC AGTTAATAGA TAGAAGAGGA ATAAGTAATA GGTCAAGACC AACGCAGCTG GTAAGTGGGG GCCTGGGATC AAATAGCTAC CTGCCTAATC CTGCCWCCTT GAGCCCTGAA TGAGTCTGCC TTCCAGGGCT CAAGGTGCTC AACAAAACAA CAGGCCGCT ATTTTCCTGG CATCTGTGCC CTGTTGGCT AGCTAGGAGC ACACATACAT AGAAATTAAA TGAAACAGAC CTTCAGCAAG GGGACAGAGG ACAGAATTAA CTTGCCAG ACACTGGAAA CCCATGTATG	2520 2580 2640 2700 2760

	AACACTCACA	TGTTTGGAA	GGGGGAAGGG	CACATGAAA	TGAGGACTCT	TCCTCATTCT	2820	
	ATGGGGCACT	CTGGCCCTGC	CCCTCTCAGC	TACTCATCCA	TCCAACACAC	CTTTCTAAGT	2880	
	ACCTCTCTCT	GCCTACACTC	TGAAGGGGTT	CAGGAGTAAC	TAACACAGCA	TCCCTTCCCT	2940	
	CAAATGACTG	ACAATCCCTT	TGTCTGCTT	TGTTTTCTT	TCCAGTCAGT	ACTGGGAAAG	3000	
5	TGGGGAAGGA	CAGTCATGGA	GAAACTACAT	AAGGAAGCAC	CTTGCCCTTC	TGCCCTCTTGA	3060	
	GAATGTTGAT	GAGTATCAA	TCTTCAAAC	TTTGGAGGTT	TGAGTAGGGG	TGAGACTCAG	3120	
	TAATGTCCT	TCCAATGACA	TGAACCTGCT	CACTCATCCC	TGGGGGCCAA	ATTGAACAAT	3180	
	CAAAGGCAGG	CATAATCCAG	TTATGAATT	TTGCGGCCGC	TTGCTAGCTT	CACGTGTTGG	3240	
10	ATCCAACCAC	GGAAAGGGCCC	TATTCTATAG	TGTCACCTAA	ATGCTAGAGC	TCGCTGATCA	3300	
	GCCTCGACTG	TGCCCCTAG	TTGCCAGCCA	TCTGTTGTTT	GCCCCTCCCC	CGTGCCTTCC	3360	
	TTGACCCCTGG	AAGGTGCCAC	TCCCACGTG	CTTCTCTAAT	AAAATGAGGA	AATTGCATCG	3420	
	CATTGTCCTG	GTAGGTGTC	TTCTATTCTG	GGGGTGGGG	TGGGGCAGGA	CAGCAAGGGG	3480	
	GAGGATTGGG	AAGACAATAG	CAGGCATGCT	GGGGATGCGG	TGGGCTCTAT	GGCTTCTGAG	3540	
15	GCGGAAAGAA	CCAGCTGGG	CTCTAGGGGG	TATCCCCAGC	CGCCCTGTAG	CGCGCATT	3600	
	AGCGCGCGG	GTGTCGTTG	TACGCGCAGC	GTGACCGCTA	CACTTGCAG	CGCCCTAGCG	3660	
	CCCGCTCCTT	TCGCTTCTT	CCCTTCCCTT	CTGCCACGT	TCGCGGGGCC	TCTCAAAAAA	3720	
	GGGAAAAAAA	GCATGCATC	CAATTAGTCA	GCAACCATAG	TCCC GCCCT	AACTCCGCC	3780	
	ATCCCGCC	TAACTCCGCC	CAGTCCGCC	CATTCTCCGC	CCCATGGCTG	ACTAATT	3840	
20	TTTATTATG	CAGAGGCCG	GGCCGCCTCG	GCCTCTGAGC	TATTCCAGAA	GTAGTGAGGA	3900	
	GGCTTTTTG	GAGGCCTAGG	CTTTTGCAAA	AAGCTTGGAC	AGCTCAGGGC	TGGGATTTCG	3960	
	CGCCAAACTT	GACGGCAATC	CTAGCGTGAA	GGCTGGTAGG	ATTTTATCCC	CGCTGCCATC	4020	
	ATGGTTCGAC	CATTGAAC	TATCGTCGCC	GTGCCCCAAA	ATATGGGGAT	TGGCAAGAAC	4080	
	GGAGACCTAC	CCTGGCCTCC	GCTCAGGAAC	GAGTCAAGT	ACTTCCAAAG	AATGACCACA	4140	
25	ACCTCTTCAG	TGGAAGGTA	ACAGAATCTG	GTGATTATGG	GTAGGAAAAC	CTGGTTCTCC	4200	
	ATTCCCTGAGA	AGAATCGACC	TTTAAAGGAC	AGAATTAATA	TAGTTCTAG	TAGAGAACTC	4260	
	AAAGAACAC	CACGAGGAGC	TCATTTCTT	GCCAAAAGTT	TGGATGATGC	CTTAAGACTT	4320	
	ATTGAACAAC	CGGAATTGGC	AAGTAAAGTA	GACATGGTT	GGATAGTCGG	AGGCAGTTCT	4380	
	GTTTACCAAG	AAGCCATGAA	TCAACCAGGC	CACCTAGAC	TCTTGTGAC	AAGGATCATG	4440	
30	CAGGAATTG	AAAGTGACAC	GT	TTTCCC	GAAATTGATT	TGGGAAATA	TAACATTCTC	4500
	CCAGAATACC	CAGGCGTCT	CTCTGAGGTC	CAGGAGGAAA	AAGGCATCAA	GTATAAGTTT	4560	
	GAAGTCTACG	AGAAGAAAGA	CTAACAGGAA	GATGCTTCA	AGTTCTCTGC	TCCCCTCCTA	4620	
	AAGCTATGCA	TTTTTATAAG	ACCATGGAC	TTTGCTGGC	TTTAGATCTC	TTTGTGAAGG	4680	
	AACCTTACTT	CTGTGGTGTG	ACATAATTGG	ACAAACTACC	TACAGAGATT	TAAGCTCTA	4740	
35	AGGTAAATAT	AAAATTTTA	AGTGTATAAT	GTGTTAAACT	ACTGATTCTA	ATTGTTGTG	4800	
	TATTTAGAT	TCCAACCTAT	GGAACGTGATG	AATGGGAGCA	GTGTTGGAA	GCCTTAAATG	4860	
	AGGAAAACCT	GT	TTTGCTCA	GAAGAAATGG	CATCTAGTG	GTATGAGGCT	4920	
	CTCAACATTC	TACTCCTCA	AAAAAGAAGA	GAAAGGTAGA	AGACCCCAAG	GACTTCTT	4980	
	CAGAATTGCT	AAGTTTTTG	AGTCATGCTG	TGTTTAGTAA	TAGAACTCTT	GCTTGCTTTG	5040	
40	CTATTACAC	CACAAAGGAA	AAAGCTGCAC	TGCTATACAA	GAAAATTATG	GAAAATATT	5100	
	CTGTAACCTT	TATAAGTAGG	CATAACAGTT	ATAATCATAA	CATACTGTTT	TTTCTTACTC	5160	
	CACACAGGCA	TAGAGTGTCT	GCTATTAATA	ACTATGCTA	AAAATTGTGT	ACCTTTAGCT	5220	
	TTTTAATTG	TAAGGGGTT	AATAAGGAAT	ATTGATGTA	TAGTGCCTT	ACTAGAGATC	5280	
	ATAATCAGCC	ATACCACATT	TGTAGAGGTT	TTACTTGCTT	AAAAAAACCT	CCACACACCTC	5340	
45	CCCTGAAACC	TGAAACATAA	AATGAATGCA	ATTGTTGTTG	TAAACTGTT	TATTGAGCT	5400	
	TATAATGGTT	ACAAATAAAG	CAATAGCATC	ACAAATTTC	CAAATAAAGC	ATTTTTTCA	5460	
	CTGCATTCTA	GTTGTTGTTT	GTCCAAACTC	ATCAATGTA	CTTATCATGT	CTGGATCGGC	5520	
	TGGATGATCC	TCCAGCGCGG	GGATCTCATG	CTGGAGTTCT	TCGCCACCC	CAACTGTTT	5580	
	ATTGCGACTT	ATAATGGTTA	CAAATAAAGC	AATAGCATCA	CAAATTTCAC	AAATAAAGCA	5640	
50	TTTTTTTCAC	TGCATTCTAG	TTGTTGTTT	TCCAAACTCA	TCAATGTATC	TTATCATGTC	5700	
	TGTATACCCT	CGACCTCTAG	CTAGAGCTT	GCGTAATCAT	GGTCATAGCT	GTTCCTGTG	5760	
	TGAAATTGTT	ATCCGCTAC	AATTCCACAC	AACATACGAG	CCGGAAGCAT	AAAGTGTAAA	5820	
	GCCTGGGGTG	CCTAATGAGT	GAGCTAAC	ACATTAATTG	CGTTGCGCTC	ACTGCCCGCT	5880	
	TTCCAGTCGG	GAAACCTGTC	GTGCCAGCTG	CATTAATGAA	TCGGCCAACG	CGCGGGGAGA	5940	
55	GGCGGTTTG	GTATTGGCG	CTCTCCGCT	TCCTCGCTCA	CTGACTCGCT	GGGCTCGGT	6000	
	GTTCGGCTGC	GGCGAGCGGT	ATCAGCTCAC	TCAAAGGCGG	TAATACGGTT	ATCCACAGAA	6060	
	TCAGGGGATA	ACGCAGGAAA	GAACATGTGA	GCAAAAGGCC	AGCAAAGGC	CAGGAACCGT	6120	
	AAAAAGGCCG	CGTTGCTGGC	GT	TTTCC	AGGCTCCGCC	CCCCTGACGA	GCATCACAAA	6180
	AATCGACGCT	CAAGTCAGAG	GTGGCGAAAC	CCGACAGGAC	TATAAAGATA	CCAGGCCTTT	6240	
	CCCCCTGGAA	GCTCCCTCGT	GCGCTCTCCT	GTTCCGACCC	TGCCGCTTAC	CGGATACCTG	6300	

	TCCGCCCTTC TCCCTTCGGG AAGCGTGGCG CTTCTCAAT GCTCACGCTG TAGGTATCTC	6360
	AGTCGGTGT AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC CGTTCAGCCC	6420
5	GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG ACACGACTTA	6480
	TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTACGAGAG CGAGGTATGT AGGCGGTGCT	6540
	ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC	6600
	TGCGCTCTGC TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTG ATCCGGCAA	6660
	CAAACCACCG CTGGTAGCGG TGGTTTTTT GTTGCAGAC AGCAGATTAC GCGCAGAAAA	6720
	AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA GTGGAACGAA	6780
10	AACTCACGTT AAGGGATTT GGTCATGAGA TTATCAAAA GGATCTTCAC CTAGATCCTT	6840
	TTAAATTAAA AATGAAGTT TAAATCAATC TAAACTATAT ATGAGTAAAC TTGGTCTGAC	6900
	AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCATT TCGITCATCC	6960
	ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT ACCATCTGGC	7020
	CCCAGTCTG CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATT ATCAGCAATA	7080
15	AACCAGCCAG CGGAAAGGGC CGAGCGCAGA AGTGGTCTG CAACTTATC CGCCTCCATC	7140
	CACTTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTAA TAGTTTGC	7200
	AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTGG TATGGCTTCA	7260
	TTCAGCTCCG GTTCCCAACG ATCAAGGCAG GTTACATGAT CCCCATGTT GTGCAAAAAA	7320
	GCGGTTAGCT CTTCCGGTCC TCCGATCGTT GTAGAGTA AGTTGGCCGC AGTGTATCA	7380
	CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT AAGATGTTT	7440
20	TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT	7500
	TGCTCTTGCC CGCGCTCAAT ACGGGATAAT ACCCGGCCAC ATAGCAGAAC TTTAAAAGTG	7560
	CTCATCATTG GAAAAGTTC TTCGGGGCGA AAACCTCTAA GGATCTTAC GCTGTTGAGA	7620
	TCCAGTTCGA TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTT TACTTTCACC	7680
	AGCGTTCTG GGTGAGCAA AACAGGAAGG CAAATGCCG CAAAAAAGGG AATAAGGGCG	7740
25	ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTCAAT ATTATTGAAG CATTATCAG	7800
	GGTTATTGTC TCATGAGCGG ATACATATT GAATGTATTT AGAAAATAA ACAAAATAGGG	7860
	GTTCCCGCAG CATTTCGGG AAAAGTGCAG CCTGACGTCG ACGGATCGGG AGATCTGCTA	7920
	GCCCCGGTGA CCTGAGGCCG GCCGGCTTCG AATAGCCAGA GTAACCTTT TTTTTAATT	7980
	TATTTTATT TATTTTTGAG ATGGAGTTTG GCGCGATCT CCCGATCCC TATGGTCGAC	8040
30	TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATCTGCTCC CTGCTTGTGT	8100
	GTTGGAGGTC GCTGAGTAGT GCGCGAGCAA AATTTAAGCT ACAACAAGGC AAGGCTTGAC	8160
	CGACAATTGC ATGAAGAAC TGCTTAGGGT TAGGCCCTTT GCGCTGCTTC GCGATGTACG	8220
	GGCCAGATAT ACGCGTTGAC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG	8280
35	GTCATTAGTT CATAGCCCAT ATATGGAGTT CGCGCTTACA TAACTTACGG TAAATGGCCC	8340
	GCCTGGTGA CGGCCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT	8400
	AGTAACGCCA ATAGGGACTT TCCATTGACG TCAATGGGT GACTATTAC GTTAAACTGCG	8460
	CCACTTGGCA GTACATCAAG TGTATCATAT GCCAAGTACG CCCCTATTG ACGTCAATGA	8520
	CGGTAATGG CCCGCGTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCTACTTG	8580
40	GCAGTACATC TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTT GGCAGTACAT	8640
	CAATGGGCGT GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT	8700
	CAATGGGAGT TTGTTTTGGC ACCAAATCA ACGGGACTTT CCAAAATGTC GTAACAACTC	8760
	CGCCCCATTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAAGCAGAGC	8820
	TCTCTGGCTA ACTAGAGAAC CCACTGCTTA CTGGCTTATC GAAATTAATA CGACTCACTA	8880
	TAGGGAGACC CAAGCTT	8897

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(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 8321 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA	60
TTGGAATTCT TGCGGCCGCT TGCTAGCCAC CATGGAGTTG TGGTTAAGCT TGGTCTTCCT	120

	TGTCTTGTT	TTAAAAGGTG	TCCAGTGTGA	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	180
	AGTCAGCCT	GGAGGGTCCC	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCACTGA	240
	CTATTACATG	TATTGGGTTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACTACAT	300
	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT	TCACCACATCTC	360
5	CAGAGACAAT	GCAAAGAACAA	GCCTGTACCT	GCAAATGAAC	AGCCTGAGGG	ACGAGGACAC	420
	AGCGGTGAT	TACTGTGCAA	GAGGCCCTGGC	GGACGGGGCC	TGGTTGCTT	ACTGGGGCCA	480
	AGGGACTCTG	GTCACGGTCT	CTTCAGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	540
	ACCCCTCTCC	AAGAGCACCT	CTGGGGCAC	AGCGGCCCTG	GGCTGCCCTG	TCAAGGACTA	600
	CTTCCCCGAA	CCGGTGACCG	TGTCGTGGAA	CTCAGGCGCC	CTGACCGAGCG	GGGTGCACAC	660
10	CTTCCCCGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC	AGCAGCGTGG	TCACCGTGCC	720
	CTCCAGCAGC	TTGGGCACCC	AGACCTACAT	CTGCAACGTG	AATCACAAGC	CCAGCAACAC	780
	CAAGGTGGAC	AAGAAAAGTTG	GTGAGAGGGC	AGCACAGGGG	GGGAGGGTGT	CTGCTGGAAG	840
	CCAGGCTCAG	CGCTCCTGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA	900
	AGGAGGCCCC	CGTCTGCCCT	TTCACCCGGA	GGCCTCTGCC	CGCCCCACTC	ATGCTCAGGG	960
15	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCC	CAGGCTAGGT	GCCCCTAACC	1020
	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	1080
	AGGACCCCTG	CCCTGACCTA	AGCCCACCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	1140
	GACACCTTCT	CTCCTCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCAAAT	1200
	CTTGTGACAA	AACTCACACAA	TGCCCACCGT	GCCCAGGTAA	GCCAGCCAG	GCCTCGCCCT	1260
20	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT	CCAGGGACAC	ACCACGTGGG	1320
	TACCAACATG	TCCGGAGGCCA	CATGGACAGA	GGCCGGCTCG	GCCCACCCCTC	TGCCCTGAGA	1380
	GTGACCGCTG	TACCAACCTC	TGTCCTACAA	GGGCAGCCCC	GAGAACACAA	GGTGTACACC	1440
	CTGCCCCCAT	CCCGGGATGA	GCTGACCAAG	AACCAGGTCA	GCCTGACCTG	CCTGGTCAAA	1500
	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAAC	1560
25	TACAAGACCA	CGCCTCCCGT	GCTGGACTCC	GACGGCTCC	TCTTCCTCTA	CAGCAAGCTC	1620
	ACCGTGGACA	AGAGCAGGTG	GCAGCAGGGG	AAACGTCTTC	CATGCTCCGT	GATGCATGAG	1680
	GCTCTGCACA	ACCACTACAC	GCAGAAAGAGC	CTCTCCCTGT	CTCCGGGTAA	ATGAGTGCAG	1740
	CGGCCGGCAA	GCCCCCGCTC	CCCAGGCTCT	CGCGGTGCGA	CGAGGATGCT	TGGCACGTAC	1800
	CCCCGTACAA	TACTTCCCAG	GCGCCCAGCA	TGGAAATAAA	GCACCCAGCG	CTGCCCTGGG	1860
30	CCCCCTGCGAG	ACTGTGATGG	TTCTTCCAC	GGGTCAAGGCC	GAGTCTGAGG	CTTGAGTGGC	1920
	ATGAGGGAGG	CAGAGCGGGT	CCCACGTGTC	CCACACTGGC	CCAGGGTGTG	CAGGTGTGCC	1980
	TGGGCCCTCT	AGGGTGGGGC	TCAGCCAGGG	GCTGCCCTCG	GCAGGGTGGG	GGATTTGCCA	2040
	GCCTGGCCCT	CCCTCCAGCA	GCACCTGCC	TGGGCTGGGC	CACGGGAAGC	CCTAGGAGCC	2100
	CCTGGGGACA	GACACACAGC	CCCTGCCTCT	GTAGGAGACT	GTCCTGTCT	GTGAGCGCCC	2160
35	CTGCTCTCCC	GACCTCCATG	CCCACCGGG	GGCATGCC	GTCCATGTGC	GTAGGGACAG	2220
	GCCCTCCCTC	ACCCATCTAC	CCCCACGGG	CTAACCCCTG	GCTGCCCTGC	CCAGCCTCGC	2280
	ACCCGATGG	GGACACAAAC	GACTCGGGGG	ACATGCACTC	TCGGGCCCTG	TGGAGGGACT	2340
	GGTGCAGATG	CCCACACACA	CACTCAGCCC	AGACCCGTTG	AAACAAACCC	GAACGTGAGGT	2400
	TGGCCGGCCA	CACGGCCACC	ACACACACAC	GTGCACGCC	CACACACGGA	GCCTCACCCCG	2460
40	GGCGAACTGC	ACAGCACCCA	GACCAAGAGC	AGGTCTCTGC	ACACGTGAAC	ACTCCTCGGA	2520
	CACAGGCCCTC	CACGAGCCCC	ACGGGCACC	TCAGGCC	CGAGCCTCTC	GGCAGCTTCT	2580
	CCACATGCTG	ACCTGCTCAG	ACAAACCCAG	CCCTCCTCTC	ACAAGGGTGC	CCCTGCAGCC	2640
	GCCACACACA	CACAGGGGAT	CACACACCAC	GTCACGTCCC	TGGCCCTGGC	CCACTTCCCA	2700
	GTGCCGCCCT	TCCCTGCGAGG	ACGGATCAGC	CTCGACTGTG	CCTTCTAGTT	GCAGGCCATC	2760
45	TGTGTGTTGC	CCCTCCCCCG	TGCCCTCCCT	GACCCGGAA	GGTGCCTACTC	CCACTGTCCT	2820
	TTCTTAATAA	AATGAGGAAA	TTGCATCGCA	TTGTCGTAGT	AGGTGTCTT	CTATTCTGGG	2880
	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGG	GGATTGGGAA	GACAATAGCA	GGCATGCTGG	2940
	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAAC	AGCTGGGGCT	CTAGGGGTA	3000
	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAAG	CGCGGCGGGT	GTGGTGGTTA	CGCGCAGCGT	3060
50	GACCGCTACA	CTTGGCCAGCG	CCCTAGCGCC	CGCTCCTTTC	GCTTTCTTCC	CTTCCTTTCT	3120
	CGCCACGTTG	GCCGGGCCCTC	TCAAAAAAGG	AAAAAAAGC	ATGCATCTCA	ATTAGTCAGC	3180
	AACCATAGTC	CCGCCCCCTAA	CTCCGCCCCAT	CCCGCCCCCTA	ACTCCGCCA	GTTCCGCCA	3240
	TTCTCCGCC	CATGGCTGAC	TAATTTTTT	TATTTATGCA	GAGGCCGAGG	CCGCCTCGGC	3300
	CTCTGAGCTA	TTCCAGAAGT	AGTGAAGGAGG	CTTTTTGGA	GGCCTAGGCT	TTTGCAAAAA	3360
55	GCTTGGACAG	CTCAGGGCTG	CGATTTCGCG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	3420
	CTGGTAGGAT	TTTATCCCG	CTGCCATCAT	GGTCAGGACA	TTGAACGTGCA	TCGTCGCCGT	3480
	GTCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCCTCCGC	TCAGGAACGA	3540
	GTTCAAGTAC	TTCCAAAGAA	TGACCCACAAC	CTCTTCAGTG	GAAGGTAAAC	AGAATCTGGT	3600
	GATTATGGGT	AGGAAAACCT	GGTCTCCAT	TCCTGAGAAG	AATCGACCTT	TAAGGACAG	3660

	AATTAATATA GTTCTCAGTA GAGAACTCAA AGAACCCACCA CGAGGGAGCTC ATTTCTTGC	3720
	CAAAAAGTTG GATGATGCCT TAAGACTTAT TGAACAAACCG GAATTGGCAA GTAAAGTAGA	3780
	CATGGTTGG ATAGTCGGAG GCAGTTCTGT TTACCAGGAA GCCATGAATC AACCAAGGCCA	3840
	CCTTAGACTC TTTGTGACAA GGATCATGCA GGAATTGAA AGTGACACGT TTTCCCAGA	3900
5	AATTGATTG GGGAAATATA AACTCTCCC AGAATACCCA GGCGTCTCT CTGAGGTCCA	3960
	GGAGGAAAAA GGCATCAAGT ATAAGTTGA AGTCTACGAG AAGAAAGACT AACAGGAAGA	4020
	TGCTTCAAG TTCTCTGCTC CCCTCTAAA GCTATGCATT TTTATAAGAC CATGGGACTT	4080
	TTGCTGGCTT TAGATCTCT TGTAAGGAA CCTTACTTCT GTGGTGTGAC ATAATTGGAC	4140
10	AAACTACCTA CAGAGATTAA AAGCTCTAAG GTAAATATAA AATTTTTAAG TGTATAATGT	4200
	GTTAAACTAC TGATTCTAAT TGTTTGTGTA TTTTAGATTG CAACCTATGG AACTGATGAA	4260
	TGGGAGCAGT GGTGGAATGC CTTTAATGAG GAAAACCTGT TTTGCTCAGA AGAAATGCCA	4320
	TCTAGTGTG ATGAGGCTAC TGCTGACTCT CAACATTCTA CTCCCTCAGA AAAGAAGAGA	4380
	AAGGTAGAAG ACCCCAAAGGA CTTTCTTCA GAATTGCTAA GTTTTTGAG TCATGCTGTG	4440
15	TTTAGTAATA GAACTCTTGC TTGCTTGTCT ATTACACCA CAAAGGAAAA AGTGCACGT	4500
	CTATACAAGA AAATTATGGA AAAATATTCT GTAACCTTTA TAAGTAGGCA TAACAGTTAT	4560
	AATCATAACA TACTGTTTT TCTTACTCCA CACAGGATA GAGTGTCTG TATTAATAAC	4620
	TATGCTCAA AATTGTGTA CTTTAGCTT TTAAATTGTA AAGGGTTAA TAAGGAATAT	4680
	TTGATGTATA GTGCTTGAC TAGAGATCAT AATCAGCCAT ACCACATTG TAGAGGTTT	4740
20	ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA TGAATGCAAT	4800
	TGTTGTTGTT AACTTGTGTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA ATAGCATCAC	4860
	AAATTTCACA AATAAAAGCAT TTTTTCACT GCATTCTAGT TGTGGTTTGT CAAACACTCAT	4920
	CAATGTATCT TATCATGTCT GGATCGGCTG GATGATCCTC CAGCGGGGG ATCTCATGCT	4980
	GGAGTTCTC GCCCACCCCCA ACTTGTGTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA	5040
25	TAGCATCACA AATTTCACAA ATAAAGCATT TTTTCACTG CATTCTAGTT GTGGTTTGTC	5100
	CAAACTCATC AATGTATCTT ATCATGTCTG TATACCGTCTG ACCTCTAGCT AGAGCTTGCG	5160
	GTAATCATGG TCATAGCTGT TTCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACCAA	5220
	CATACGAGCC GGAAGCATAA AGTGTAAAGC CTGGGGTGC TAATGAGTGA GCTAACTCAC	5280
	ATTAATTGCG TTGCGCTCAC TGCCCCCTTT CCAGTCGGGA AACCTGTCGT GCCAGCTGCA	5340
30	TTAATGAATC GGCCAACCGCG CGGGGAGAGG CGGTTTGCCT ATTGGGCCT CTTCCGCTTC	5400
	CTCGCTCACT GACTCGCTGC GCTCGTCTGT TCGGCTGCG CGAGCGTAT CAGCTCACTC	5460
	AAAGCGGTA ATACGGTTAT CCACAGAAC AGGGGATAAC GCAGGAAAGA ACATGTGAGC	5520
	AAAAGGCCAG CAAAAGGCCA GGAACCGTAA AAAGGCCCGG TTGCTGGCGT TTTCCATAG	5580
	GCTCCGCCCG CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GCGAAACCC	5640
35	GACAGGACTA TAAAGATACC AGGCCTTCC CCCTGGAAAGC TCCCTCGTGC GCTCTCCTGT	5700
	TCCGACCCCTG CCGCTTACCG GATACTCTG CCGCTTCTCTC CCTTCGGAA GCGTGGCGCT	5760
	TTCTCAATGC TCACGCTGTA GGTATCTCG TTGCTGTAG GTCTGTTCGCT CCAAGCTGGG	5820
	CTGTGTGCAC GAACCCCCCG TTCAGCCCCG CCGCTGCGCC TTATCCGGTA ACTATCGTCT	5880
	TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCACTGGCA GCAGCCACTG GTAACAGGAT	5940
40	TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC CTAACATCGG	6000
	CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA	6060
	AAGAGTTGGT AGCTCTTGAT CGGCCAAACAA AACCAACCGCT GGTAGCGGTG GTTTTTTGT	6120
	TTGCAAGCAG CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTT	6180
	TACGGGGTCT GACGCTCAGT GGAAGAAAAA CTCACGTTAA GGGATTGTTGG TCATGAGATT	6240
	ATCAAAAAGG ATCTTCACCT AGATCTTTT AAATTAAAAA TGAAGTTTA AATCAATCTA	6300
45	AAGTATATAT GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT	6360
	CTCAGCGATC TGTCTATTTG GTCATCCAT AGTTGCCTGA CTCCCGCTCG TGTAGATAAC	6420
	TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG	6480
	CTCACCGGCT CCAGATTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG AGCCAGAAG	6540
	TGGTCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAT TGTGCGGGG AAGCTAGAGT	6600
50	AAGTAGTTCG CCAGTTATA GTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT	6660
	GTCACGCTCG TCGTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCAGT	6720
	TACATGATCC CCCATGTTGT GCAAAAAAGC GTTGTAGCTCC TTCGGTCTC CGATCGTTGT	6780
	CAGAAGTAAG TTGGCCGAG TGTTATCACT CATGGTTATG GCAGCACTGC ATAATTCTCT	6840
	TACTGTCATG CCATCCGTAA GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT	6900
55	CTGAGAATAG TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAATAC GGGATAATAC	6960
	CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGCGAAA	7020
	ACTCTCAAGG ATCTTACCGC TGTTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA	7080
	CTGATCTTCA GCATTTTTA CTTTACCCAG CGTTTCTGGG TGAGAAAAA CAGGAAGGCA	7140
	AAATGCCGCA AAAAGGGAA TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCT	7200

	TTTTCAATAT TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTG	7260
	ATGTATTTAG AAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC	7320
	TGACGTCGAC GGATCGGGAG ATCTGCTAGG TGACCTGAGG CGCGCCGGCT TCGAATAGCC	7380
5	AGAGTAACCT TTTTTTTAA TTTTATTTA TTTTATTTT GAGATGGAGT TTGGCGCCGA	7440
	TCTCCCAGTC CCCTATGGTC GACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAC	7500
	CAGTATCTGC CCCCTGCTTG TGTGTTGGAG GTGCGCTGAGT AGTGCAGCAG CAAAATTAA	7560
	GCTACAACAA GGCAAGGCTT GACCGACAAT TCATGAAGA ATCTGCTTAG GGTTAGGC	7620
	TTTGCCTGC TTGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT	7680
10	TATTAATAGT AATCAATTAC GGGGTCTTA GTTCATAGCC CATATATGGA GTTCCCGCTT	7740
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	TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGG CTTTCCATTG ACGTCATGG	7860
	GTGGACTATT TAGGGTAAAC TGCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT	7920
	ACGCCCCCTA TTGACGTCAA TGACGGTAA TGCCCGCCCT GGCATTATGC CCAGTACATG	7980
15	ACCTTATGGG ACTTTCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG	8040
	GTGATGCGGT TTGGCAGTA CATCAATGGG CCTGGATAGC GGTTTGACTC ACAGGGATT	8100
	CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTGTT GGCACCAAAA TCAACGGGAC	8160
	TTTCCAAAAT GTCGTAACAA CTCCGGCCCA TTGACGCAA TGGGCGTAG GCGTGTACGG	8220
	TGGGAGGTCT ATATAAGCAG AGCTCTCTGG CTAACTAGAG AACCCACTGC TTACTGGCTT	8280
20	ATCGAAATTAA ATACGACTCA CTATAGGGAG ACCCAAGCTT G	8321

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8897 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

	GACGGATCGG GAGATCTGCT AGCCCCGGTG ACCTGAGGCC CGCCGGCTTC GAATAGCCAG	60
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	GTATCTGCTC CTCGCTTGTG TGTTGGAGGT CGCTGAGTAG TGCGCGAGCA AAATTAAAGC	240
	TACAACAAGG CAAGGCTTGA CCGACAATTG CATGAAGAAAT CTGCTTAGGG TTAGGC	300
	TGGCGCTT CGCGATGTAC GGGCCAGATA TACGCGTTGA CATTGATTAT TGACTAGTTA	360
	TTAATAGTAA TCAATTACGG GGTCAATTAGT TCATAGCCCA TATATGGAGT TCCGCGTTAC	420
40	ATAACTTACG TAAATGGCC CGCCTGGCTG ACCGCCAAC GACCCCCGCC CATTGACGTC	480
	AATAATGACG TATGTTCCA TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAATGGGT	540
	GGACTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA TGCCAAGTAC	600
	GCCCCCTATT GACGTCAATG ACGGTAATG GCCCGCCTGG CATTATGCCC AGTACATGAC	660
	CTTATGGAC TTTCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT	720
45	GATGCGTTT TGGCAGTACA TCAATGGCG TGGATAGCGG TTTGACTCAC GGGGATTCC	780
	AAGTCTCCAC CCCATTGACG TCAATGGAG TTGTTTTGG CACCAAAATC AACGGGACTT	840
	TCCAAAATGT CGTAACAACCT CGGCCATT GACGCAAATG GGCGTAGGC GTGTACGGTG	900
	GGAGGTCTAT ATAAGCAGAG CTCTCTGGCT AACTAGAGAA CCCACTGCTT ACTGGCTTAT	960
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50	TCCTTAGGTC TCGAGCACCA TGAAGTTGCC TGTTAGGCTG TTGGTGTGTA TGTTCTGGAT	1080
	TCCTGCTTCC AGCAGTGTATG TTGTCATGAC CAAACCCCA CTGTCAGTC CTGTCACGCT	1140
	TGGACAACCT CGGTCCATCT CTTGCAGATC TAGTCAGATC ATTGTACATA ATAATGGCAA	1200
	CACCTATCTG GAATGGTACC AGCAGAGACC AGGGCAGTCT CCACGGCTCC TGATCTACAA	1260
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	GGGTTCACAT GTTCCATTCA CGTTCCGCCA AGGGACAAAG TTGGAAATCA AACGTAAGTC	1440
	TCGAGTCTCT AGATAACCGG TCAATCGATT GGAATTCTAA ACTCTGAGGG GGTGGATGA	1500
	CGTGGCCATT CTTTGCTAA AGCATTGAGT TTACTGCAAG GTCAGAAAAG CATGCAAAGC	1560
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	GTGGAAGGTG GATAACGCC TCCAATCGGG TAACCTCCAG GAGAGTGTCA CAGAGCAGGA	1980
	GAGCAAGGAC AGCACCTACA GCCTCAGCAG CACCCGTGAGC CTGAGCAAAG CAGACTACGA	2040
	GAAACACAAA GTCTACGCT GCGAAGTCAC CCATCAGGGC CTGAGCTGC CCGTCACAAA	2100
	GAGCTTCAAC AGGGGAGAGT GTTAGAGGGA GAAGTGCCTCC CACCTGCTCC TCAGTTCCAG	2160
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	TCATTTATA ATTATTATCT GTTGTGTTTAC CAACTACTA ATTTCTCTTA TAAGGGACTA	2400
	AATATGTAGT CATCCTAACCG CACGTAACCA TTATATAAAA TCATCCTCA TTCTATTTA	2460
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	TAATCCACAC TATACTGTGA GATTAAAAAC ATTCACTAAA ATGTTGCAAA GGTTCTATAA	2940
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55	CCGCCTCGGC CTCTGAGCTA TTCCAGAAGT AGTGAGGAGG CTTTTTGGA GGCCCTAGGCT	4920
	TTTGCAAAAA GCTTGGACAG CTCAGGGCTG CGATTTCGCG CCAAACCTGAA CGCAATCCT	4980
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	TCGTCGCCGT GTCCCAAAAT ATGGGGATTG GCAAGAACGG AGACCTACCC TGGCCTCCGC	5100
	TCAGGAACGA GTTCAAGTAC TTCCAAAGAA TGACCACAAAC CTCTTCAGTG GAAGGTAAC	5160

	AGAATCTGGT	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT	TCCTGAGAAG	AATCGACCTT	5220
	TAAAGGACAG	AATTAATATA	GTTCTCAGTA	GAGAACTCAA	AGAACCAACCA	CGAGGAGCTC	5280
5	ATTTCTTGC	CAAAAGTTG	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	5340
	GTAAAGTAGA	CATGGTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC	5400
	AACCAGGCCA	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAAATTGAA	AGTGACACGT	5460
	TTTCCCAGA	AATTGATTG	GGGAAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCCTCT	5520
	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT	ATAAGTTGA	AGTCTACGAG	AAGAAAGACT	5580
	AACAGGAAGA	TGCTTTCAAG	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	5640
10	CATGGGACTT	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	5700
	ATAATTGGAC	AAACTACCTA	CAGAGATTAA	AACTCTAAAG	GTAAATATAA	AATTTTTAAG	5760
	TGTATAATGT	GTAAAACATC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATTG	CAACCTATGG	5820
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	AGAAATGCCA	TCTAGTGTG	ATGAGGCTAC	TCCTGACTCT	CAACATTCTA	CTCCCTCCAAA	5940
	AAAGAAAGAGA	AAGGTAGAAG	ACCCCAAGGA	CTTCTCTCA	GAATTGCTAA	GTTTTTGAG	6000
15	TCATGCTGT	TTTAGTAATA	GAACCTTTGC	TTGCTTTGCT	ATTTACACCA	CAAAGGAAAA	6060
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	TAACAGTTAT	AATCATAACA	TACTGTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	6180
	TATTAATAAC	TATGCTCAA	AATTGTGTAC	CTTTAGCTTT	TTAATTGTA	AAGGGGTTAA	6240
	TAAGGAATAT	TTGATGTATA	GTGCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTTG	6300
20	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	6360
	TGAATGCAAT	TGTTGTTGTT	AACTTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA	6420
	ATAGCATCAC	AAATTTCACA	AATAAAGCAT	TTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	6480
	CCAAACTCAT	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	6540
	ATCTCATGCT	GGAGTTCTTC	GCCCCCCCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	6600
25	AATAAAAGCAA	TAGCATCACA	AATTCACAA	ATAAAGCATT	TTTTTCACTG	CATTCTAGTT	6660
	GTGGTTTGT	CAAACTCATC	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	6720
	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT	TTCCGTGTTG	AAATTGTTAT	CCGCTCACAA	6780
	TTCCACACAA	CATACGAGCC	GGAAAGCATAA	AGTGTAAAGC	CTGGGGTGC	TAATGAGTGA	6840
	GCTAACTCAC	ATTAATTGCG	TTGCCTCCTAC	TGCCCCGCTT	CCAGTCGGGA	AACCTGTCGT	6900
30	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	GGGGGAGAGG	CGGTTTGC	ATTGGGCGCT	6960
	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT	TCGGCTGCG	CGAGCGGTAT	7020
	CAGCTCACTC	AAAGGCGGT	ATACGGTTAT	CCACAGAAC	AGGGGATAAC	GCAGGAAAGA	7080
	ACATGTGAGC	AAAAGGCCAG	CAAAAGGCCA	GGAACCGTAA	AAAGGGCG	TTGCTGGCGT	7140
	TTTCCCATAG	GCTCCGCC	CTGTGAGG	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	7200
35	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCCTTTCC	CCCTGAAAGC	TCCCTCGTGC	7260
	GCTCTCCTGT	TCCGACCCCTG	CCGCTTACCG	GATACCTGTC	CGCCTTCTC	CCTTCGGGAA	7320
	GCCTGGCGCT	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCGGTGTTAG	GTCGTTCGCT	7380
	CCAAGCTGGG	CTGTGTGCAC	GAACCCCCCG	TTCAAGCCGA	CCGCTGCG	TTATCCGGTA	7440
	ACTATCGTCT	TGAGTCCAA	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	7500
40	GTAACAGGAT	TAGCAGAGCG	AGGTATGTAG	GGCGTGTAC	AGAGTTCTT	AAGTGGTGGC	7560
	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAAGCCAGTTA	7620
	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGTAT	CCGGCAAAACA	AACCACCGCT	GGTAGCGGTG	7680
	GTTCCTTGT	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	7740
	TGATCTTTTC	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTGG	7800
45	TCATGAGATT	ATCAAAAGG	ATCTCACCT	AGATCCTTT	AAATTAAAAA	TGAAGTTTTA	7860
	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTATCAGTG	7920
	AGGCACCTAT	CTCAGCGATC	TGTCTATTT	GTTCATCCAT	AGTTGCTGA	CTCCCCGTG	7980
	TGTAGATAAC	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGTGCA	ATGATACCGC	8040
	GAGACCCACG	CTCACCGGCT	CCAGATTAT	CAGCAATAAA	CCAGCCAGCC	GGAAAGGGCG	8100
50	AGCCAGAAAG	TGGCTCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAA	TGTTGCCGGG	8160
	AAGCTAGAGT	AAGTAGTTCG	CCAGTTAATA	GTGGCGCAA	CGTTGTTGCC	ATTGCTACAG	8220
	GCATCGTGGT	GTCACGCTCG	TCGTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACCGAT	8280
	CAAGGCGAGT	TACATGATCC	CCCATGTTGT	GCAGAAAAAGC	GGTTAGCTCC	TTCGGTCCTC	8340
	CGATCGTTGT	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC	8400
55	ATAATTCTCT	TACTGTCTAT	CCATCCGTA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	8460
	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG	CTCTGCCCC	GGTCATGAA	8520
	GGGATAATAC	CGCGCCACAT	AGCAGAACTT	TAAAAGTGT	CATCATTGGA	AAACGTTCTT	8580
	CGGGCGAAA	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTGATG	TAACCCACTC	8640
	GTGCACCCAA	CTGATCTTCA	GCATTTTTA	CTTTCACCA	CGTTCTGGG	TGAGCAAAAA	8700

CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	8760
TACTCTTCCCT	TTTCATAT	TATTGAAGCA	TTTATCAGGG	TTATTGTCTC	ATGAGCGGAT	8820
ACATATTGAA	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA	8880
AAGTGCCACC	TGACGTC					8897

What is claimed is:

1. A method for inhibiting immunoglobulin-induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering an immunoglobulin molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited.
10
2. A method for inhibiting immunoglobulin-induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering a structurally altered antibody to the subject, the structurally altered antibody comprising a variable region and a constant region, multiple toxicity associated domains in the constant region being modified so as to render the constant region unable to mediate an ADCC response or activate complement thereby inhibiting immunoglobulin-induced toxicity resulting from immunotherapy.
15
3. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein having multiple structurally altered toxicity associated domains in the constant region.
20
4. A method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an Ig fusion protein to the subject, the Ig fusion protein comprising a modified constant region, the
25

modification being a structural alteration in multiple toxicity associated regions within the CH₂ domain.

5. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
 - (a) selecting an immunoglobulin which recognizes and binds a target, the target being associated with the disease;
 - 10 (b) mutating the immunoglobulin so selected by structurally altering multiple toxicity associated domains in the constant region of the immunoglobulin thereby creating a structurally altered immunoglobulin;
 - 15 (c) administering the structurally altered immunoglobulin of step (b) to the subject under conditions so that the structurally altered immunoglobulin recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the constant region thereby preventing immunoglobulin-induced toxicity in the subject.
- 20
6. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
 - (a) selecting an Ig fusion protein which recognizes and binds a target, the target being associated with the disease;
 - 25 (b) structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected;

- (c) administering the structurally altered Ig fusion protein of step (b) to the subject under conditions so that the structurally altered Ig fusion protein recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the CH₂ domain thereby preventing immunoglobulin-induced toxicity in the subject.
- 5
7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the portion of the constant region is the CH₂ domain.
- 10
8. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgG.
9. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgM.
- 15
10. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgA.
11. The method of claim 2, wherein the antibody recognizes and binds Le^y.
- 20 12. The method of claim 2, wherein the antibody recognizes and binds to Le^x.
13. The method of claim 2, wherein the antibody is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 25
14. The method of claim 2, wherein the antibody is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.

15. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds Le^y.
16. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds to Le^x.
5
17. The method of claim 1 or 5, wherein the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
10
18. The method of claim 1 or 5, wherein the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC..
15
19. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds Le^y.
20
20. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds to Le^x.
20
21. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
25
22. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
25
23. A pharmaceutical composition comprising a pharmaceutically effective

amount of a structurally altered immunoglobulin, and an acceptable carrier, the structurally altered immunoglobulin (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.

- 5 24. A pharmaceutical composition comprising a pharmaceutically effective amount of structurally altered Ig fusion protein, and an acceptable carrier, the structurally altered Ig fusion protein (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.
- 10 25. A method of treating carcinomas in vivo comprising administering to a subject a pharmaceutically effective amount of the composition of claim 23 or 24.
- 15 26. The method of claim 30, wherein the structurally altered immunoglobulin in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 20 27. The method of claim 24, wherein the Ig fusion protein in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.
- 25 28. The method of claim 2 or 5, wherein the antibody is conjugated to a cytotoxic agent.
29. The method of claim 1, wherein the immunoglobulin is conjugated to a cytotoxic agent.

30. The method of claim 3, 4 or 6, wherein the Ig fusion protein is conjugated to a cytotoxic agent.
31. The method of claim 28, 29, or 31, wherein the cytotoxic agent is selected from the group consisting of antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents.
5
32. A method for treating a subject suffering from a cancer, the cancer being characterized as a group of cells having a tumor associated antigen on the cell surface, which method comprises administering to the subject a cancer killing amount of the composition of claim 23 or 24 joined to a cytotoxic agent under conditions which permit the molecule so joined to bind the tumor associated antigen on the cell surface so as to kill the cells so bound thereby curing the subject.
10
33. A pharmaceutical composition comprising a pharmaceutically effective amount of a structurally altered BR96 antibody, the structurally altered antibody having an inactivated CH₂ domain.
15
34. A method for treating a subject suffering from a proliferative type disease characterized by cells having a BR96 antigen on the cell surface which comprises administering to the subject an effective amount of the composition of claim 33 joined to doxorubicin such that the immunoconjugate binds the BR96 antigen and kills said cells thereby treating the subject.
20
35. A method for inhibiting BR96 (ATCC: HB10036) induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering
25

BR96 to the subject, the BR96 molecule being modified prior to administration, the modification comprising the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated toxicity is inhibited.

36. A method for preventing BR96 (ATCC: HB10036) induced toxicity resulting from immunotherapy for cancer in a subject comprising:

(a) mutating the BR96 polypeptide by the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated immunoglobulin-induced toxicity is inhibited in the altered BR96 polypeptide; and

(b) administering the structurally altered BR96 polypeptide of step (a) to the subject under conditions so that the peptide recognizes and binds cancer associated Le^y antigens, thereby alleviating symptoms associated with the cancer, the structural alteration of the toxicity associated domains thereby preventing BR96 toxicity in the subject.

37. A chimeric BR96 antibody having a structurally altered constant region having the CH1 and CH3 domains but not the CH2 domain, the antibody being designated cBR96-A.

38. The chimeric BR96 antibody of claim 37 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 10.
39. A BR96 antibody having humanized variable and constant regions, wherein
5 the constant region has been structurally altered so that the CH1 and CH3 domains are present but the CH2 domain is not, the antibody being designated hBR96-2A.
40. The BR96 antibody of claim 39 which is expressed by the plasmid having
10 the sequence shown in SEQ ID NO. 12.
41. A BR96 antibody designated hBR96-2B having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine and glycine at amino acid position 237 is mutated to alanine.
15
42. A BR96 antibody designated hBR96-2C having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.
20
43. A BR96 antibody designated hBR96-2D having a structurally altered constant region wherein proline at amino acid position 331 is mutated to alanine.
- 25 44. A BR96 antibody designated hBR96-2E having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid

position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

45. A BR96 antibody designated hBR96-2F having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; and proline at amino acid position 331 is mutated to alanine.
5
46. A BR96 antibody designated hBR96-2G having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
10
- 15 47. A BR96 antibody designated hBR96-2H having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
20
48. A nucleic acid molecule which encodes the BR96 antibody of claim 37, 39, and 41-47.
25
49. A cDNA of claim 48.
50. A plasmid which comprises the nucleic acid molecule of claim 48.

51. A host vector system comprising a plasmid of claim 50 in a suitable host cell.
52. A method for producing a protein comprising growing the host vector system of claim 51 so as to produce the protein in the host and recovering the protein so produced.
5

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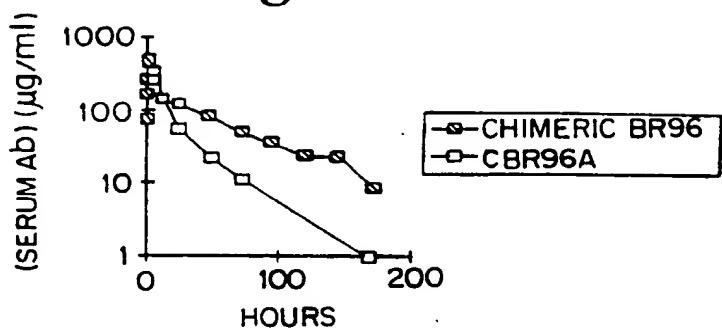
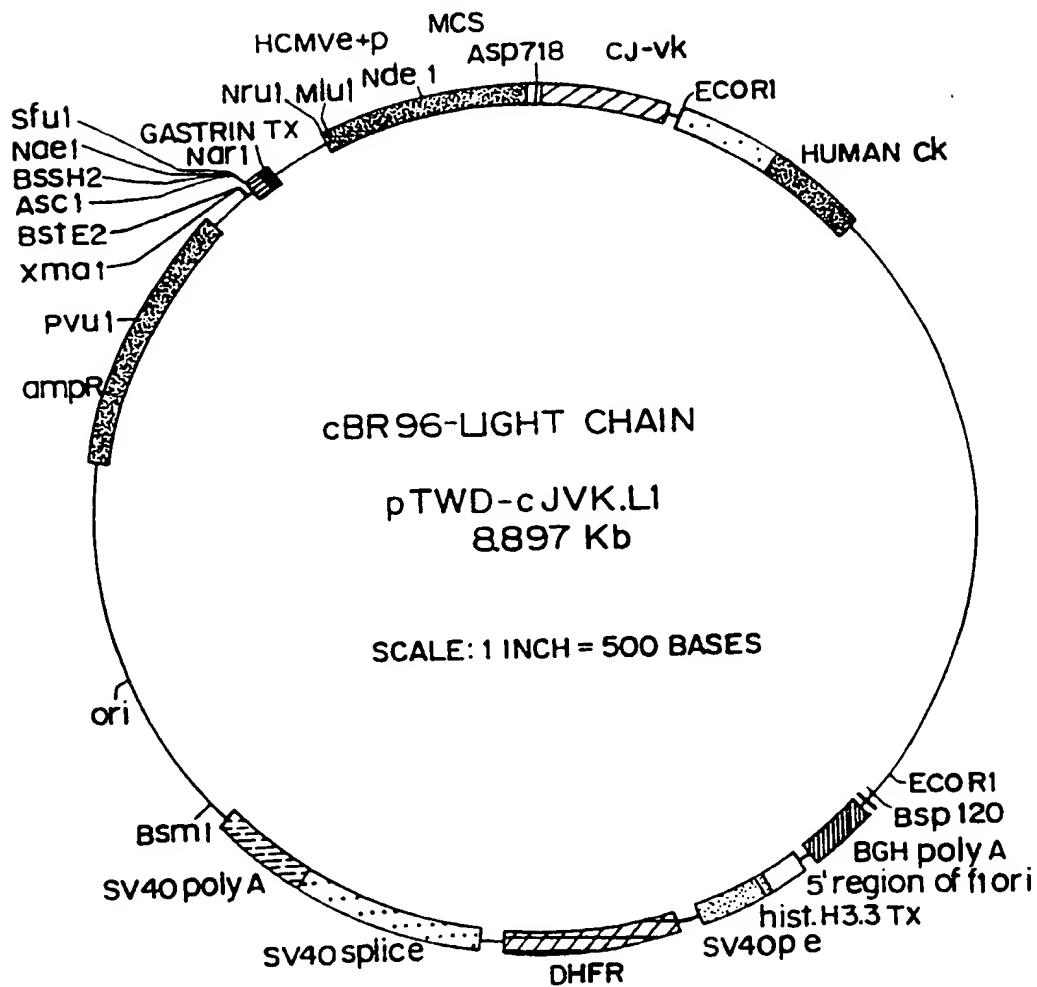
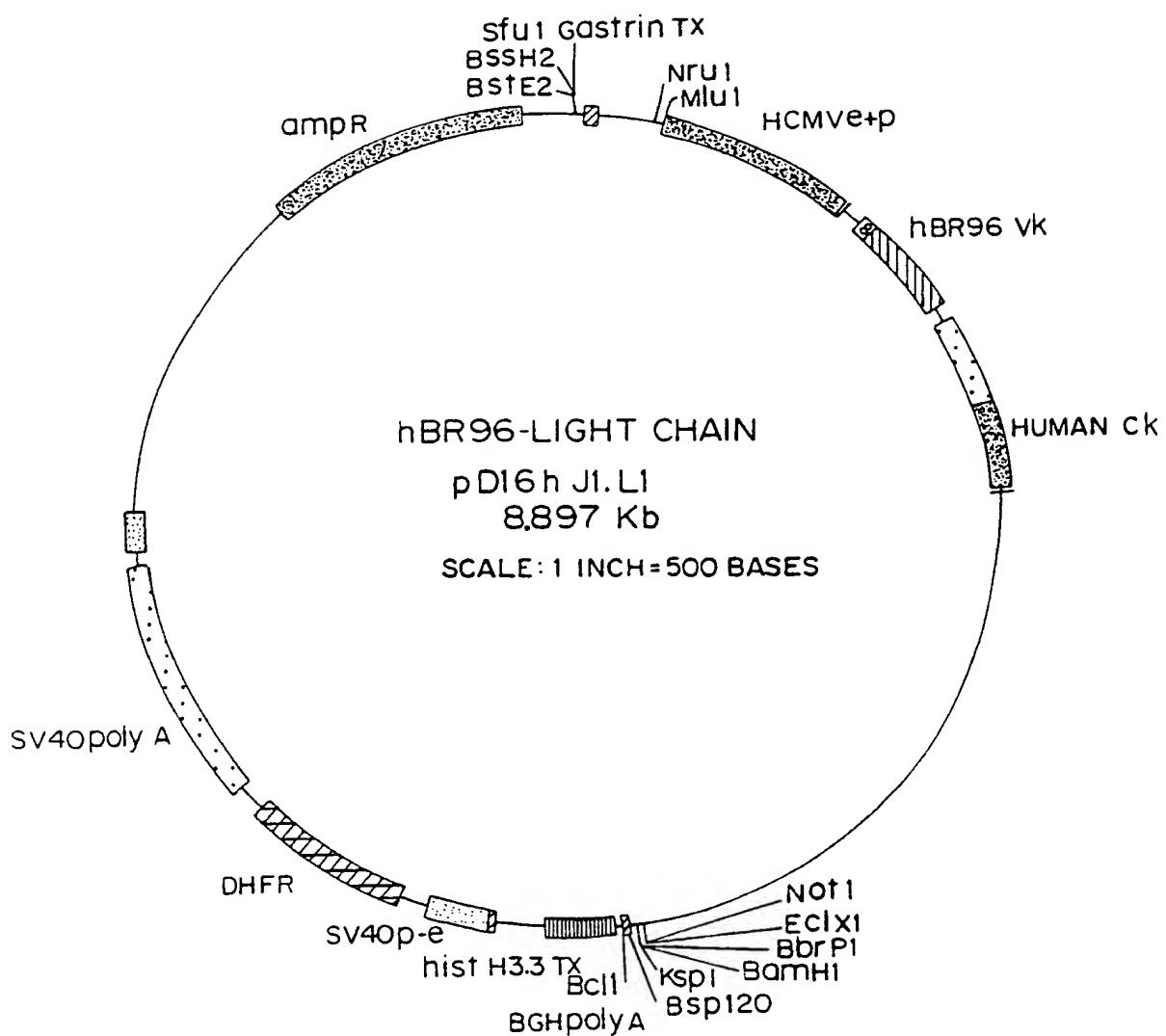
Fig. 1**Fig. 2**

Fig. 3.



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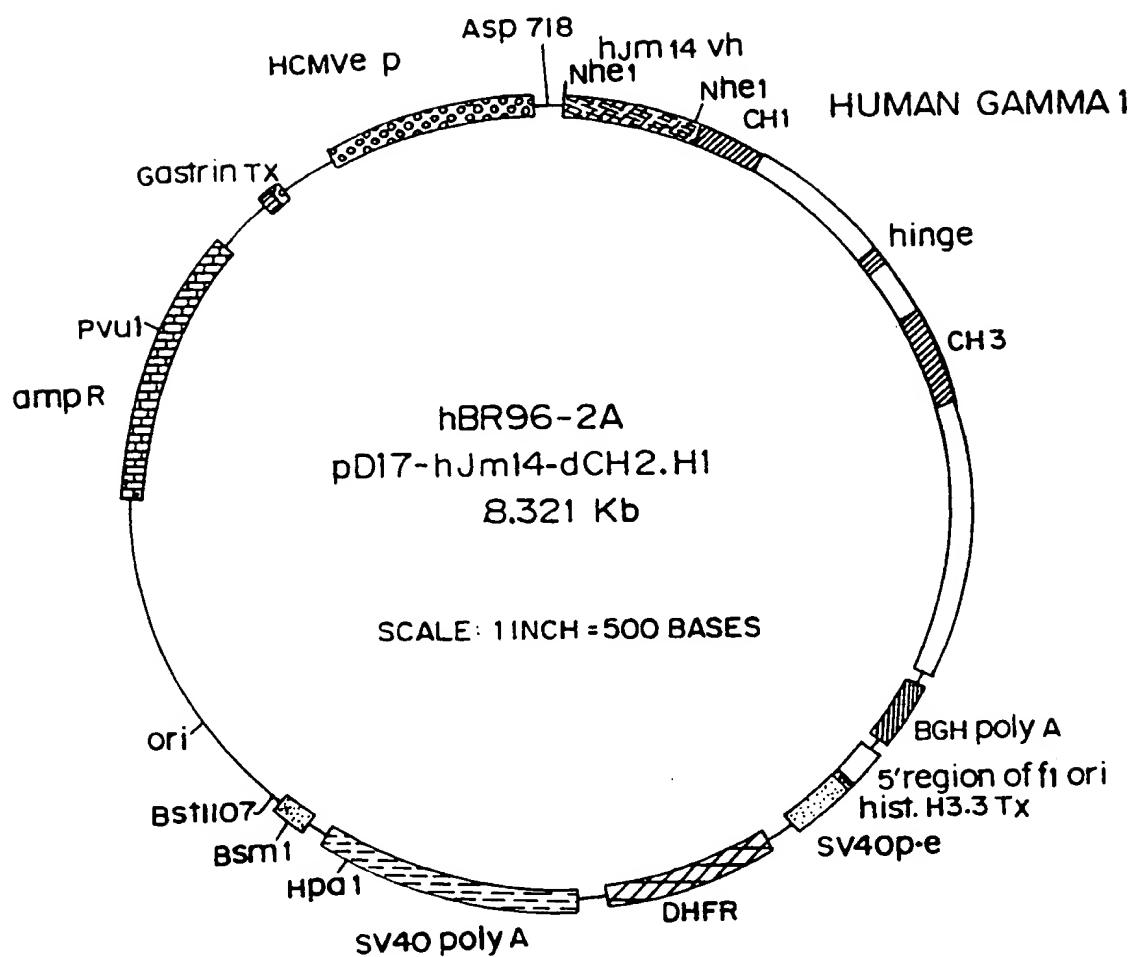
Fig. 4

Fig. 5

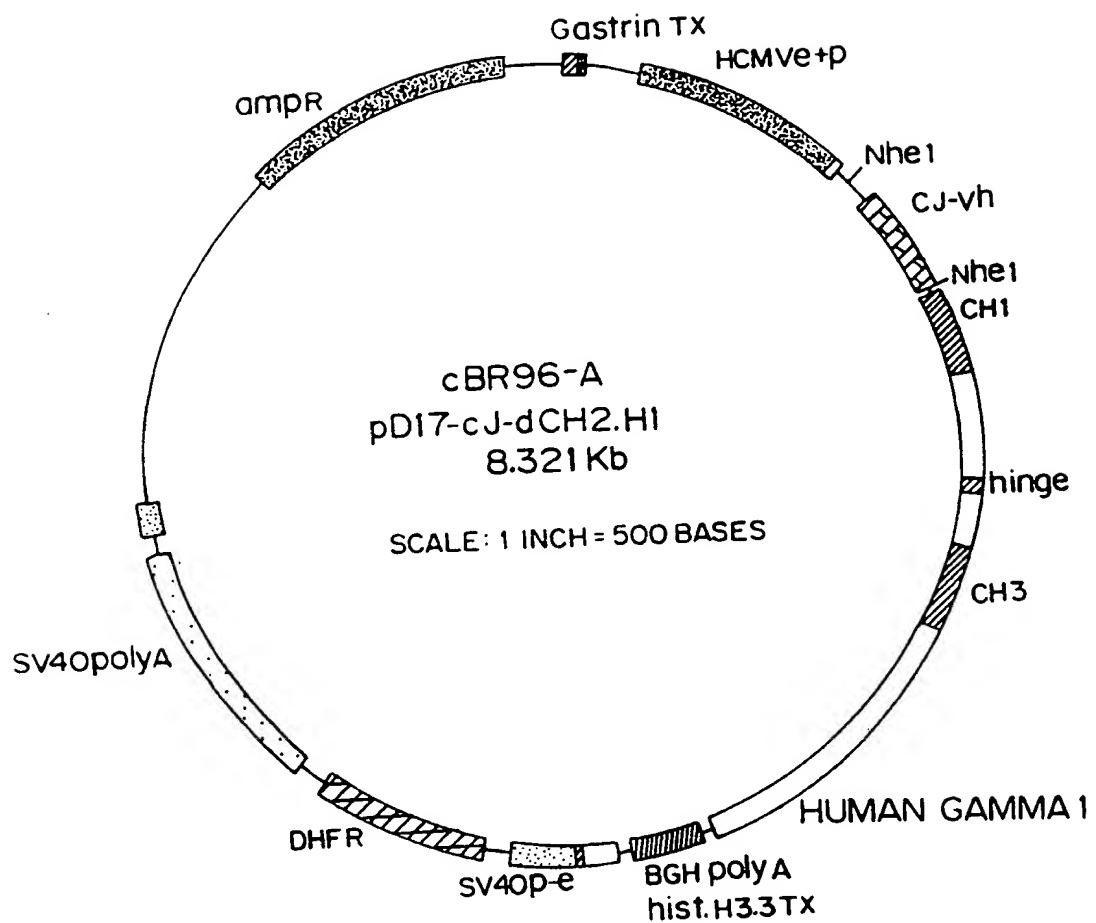
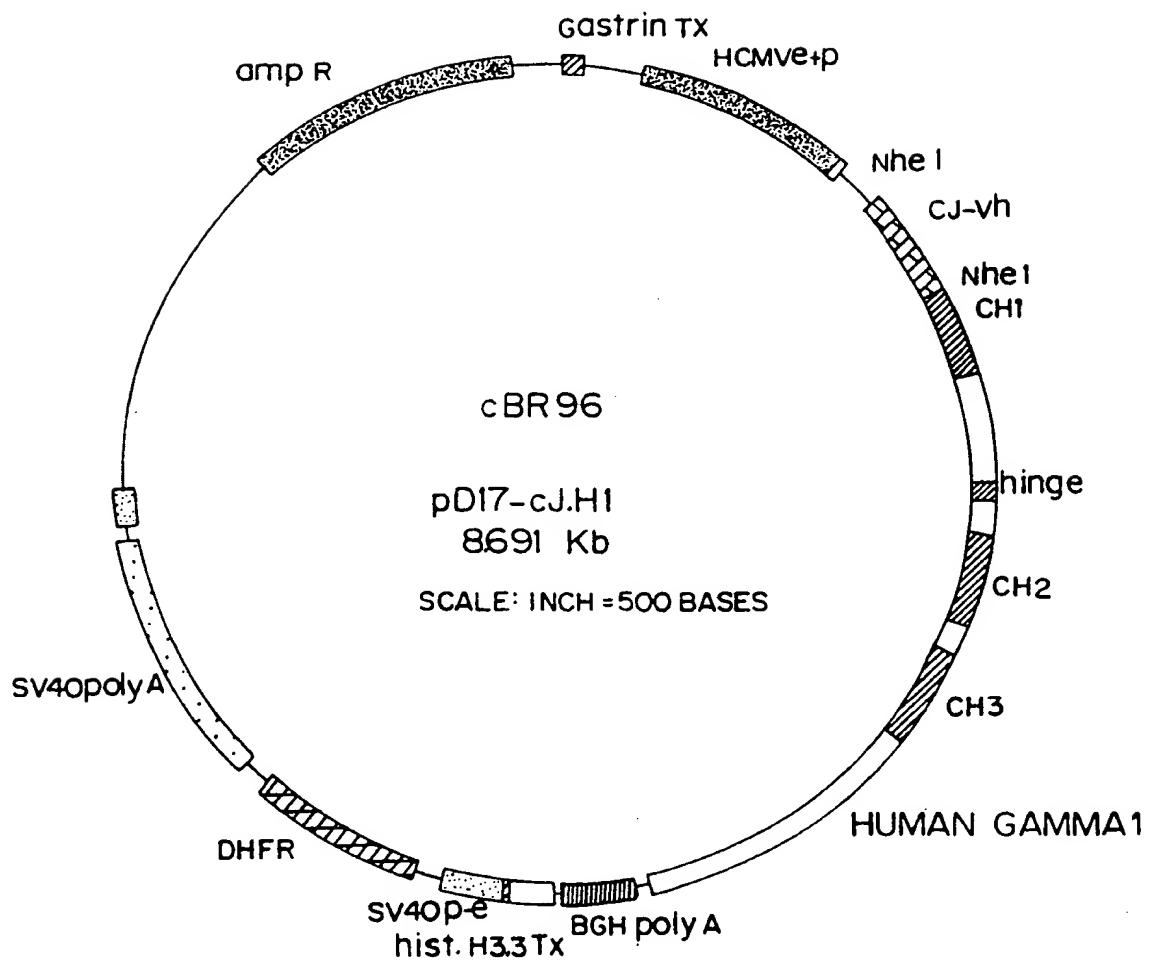
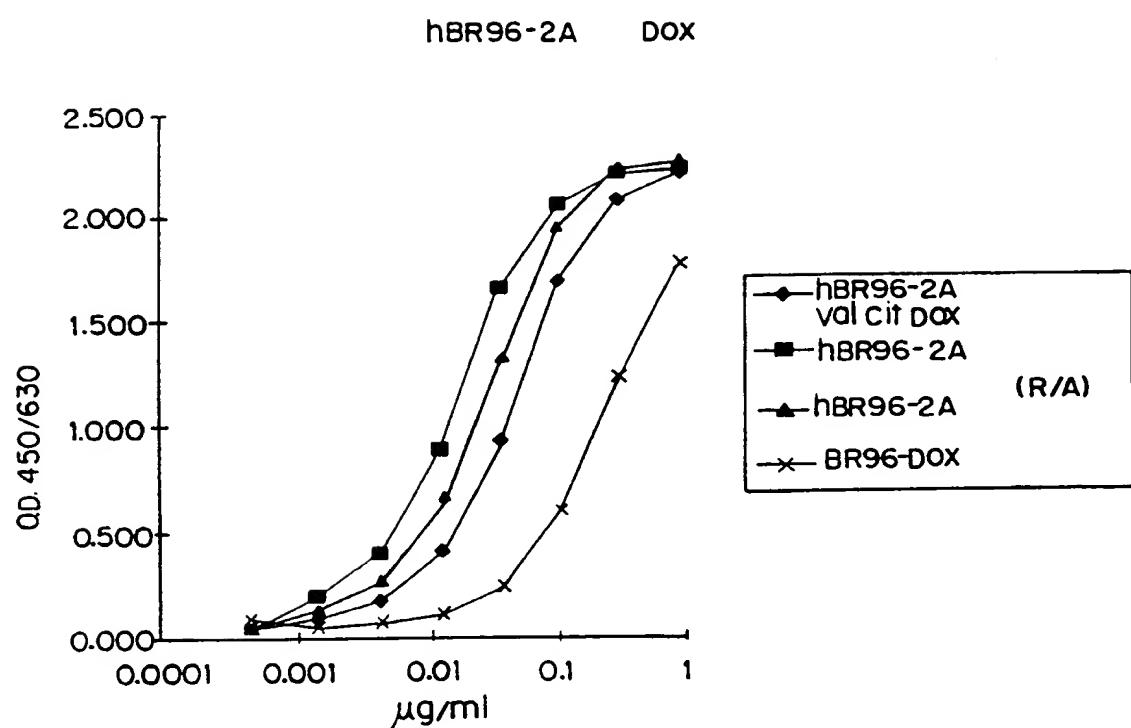
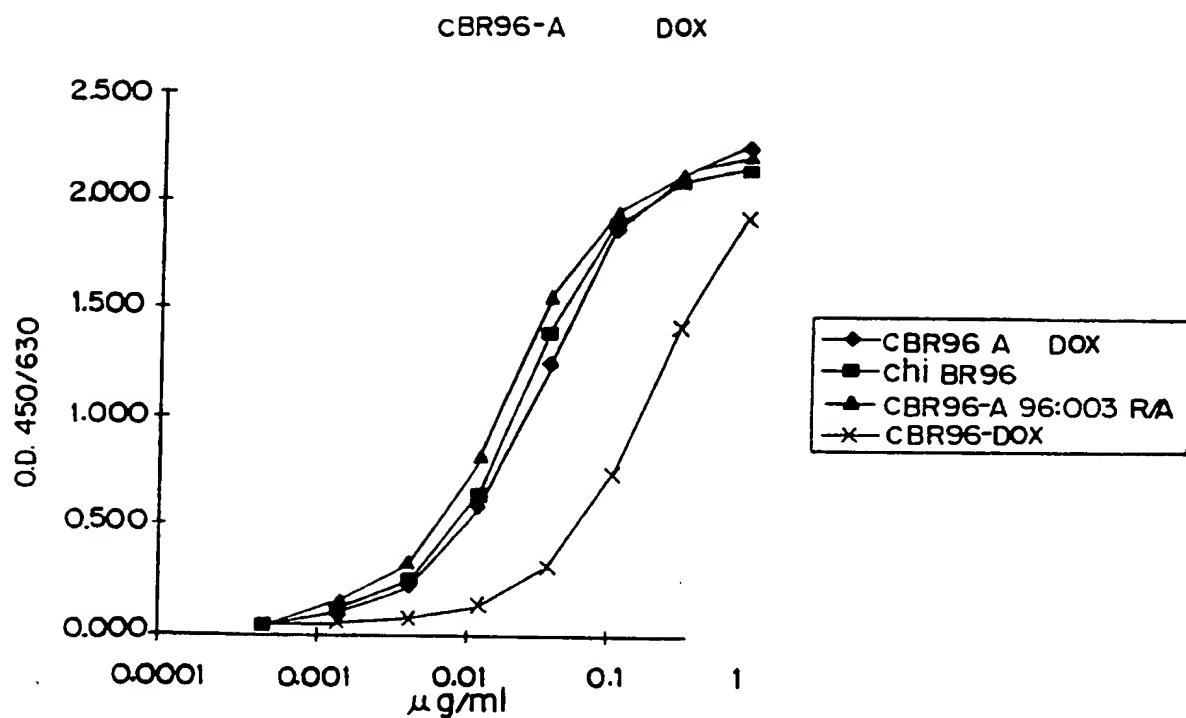


Fig. 6

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Fig. 7

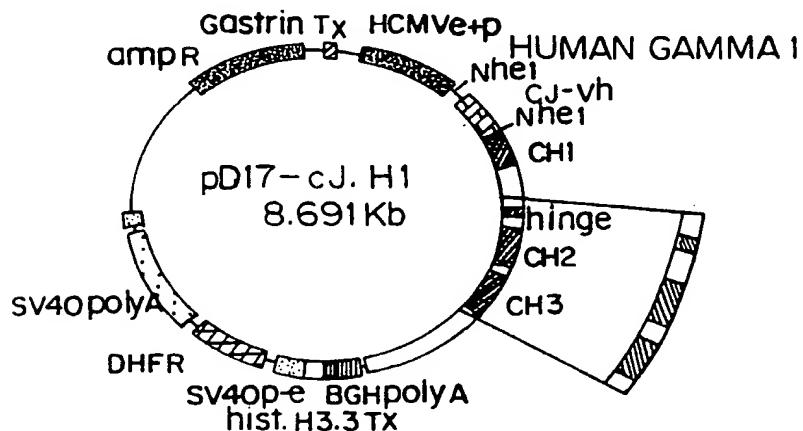
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Fig. 8

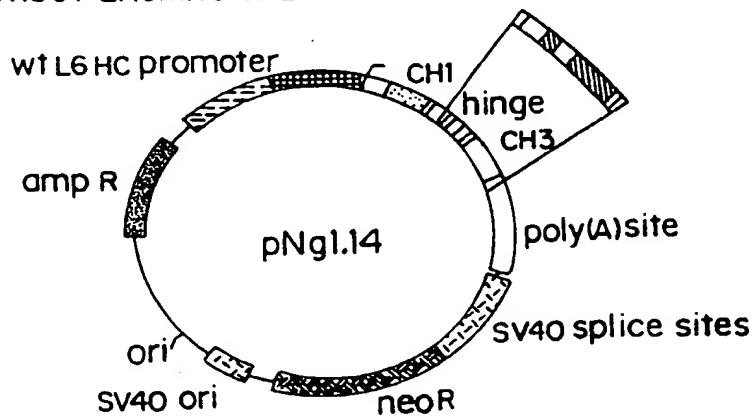
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Fig. 9A

A.-HINGE + CH2+CH3 DOMAINS WERE REMOVED FROM BR96 IgG1 CONSTRUCT BY E.CO.47-III RESTRICTION DIGESTION.

***Fig. 9B***

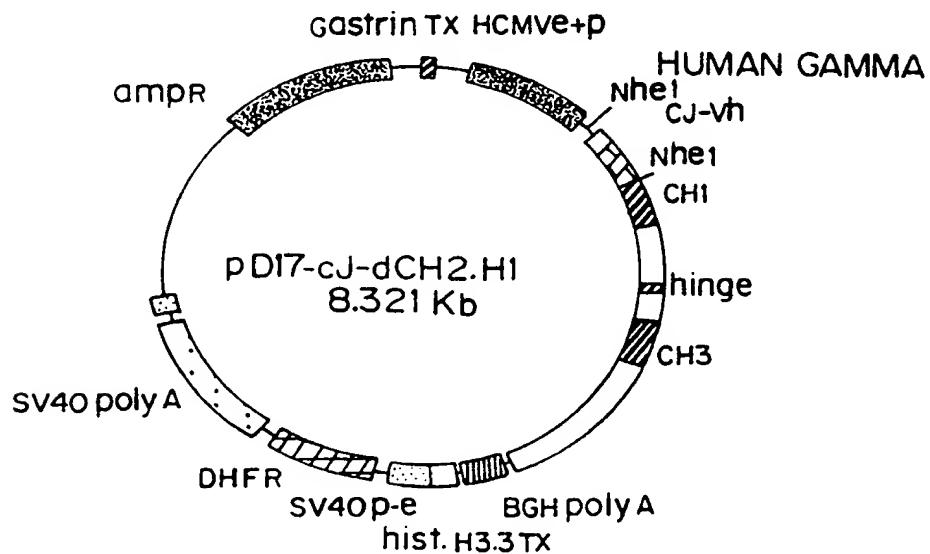
B.-HINGE+CH3 DOMAINS AMPLIFIED BY PCR FROM L6 IgG1 CONSTRUCT LACKING THE CH2 DOMAIN.



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Fig. 9C

C-HINGE+CH3 PCR FRAGMENT CLONED BY HOMOLOGOUS RECOMBINATION INTO E.CO.47-III SITE OF BR96 IgG1 MOLECULE.



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1.- INTRODUCTION OF MUTATIONS BY SITE DIRECTED
MUTAGENESIS ON DOUBLE-STRANDED PLASMID DNA.

Fig. 10A

A.- MUTATIONS INTRODUCED INTO SYNTHETIC OLIGONUCLEOTIDES
USED FOR THE PCR AMPLIFICATION OF CH2 DOMAIN

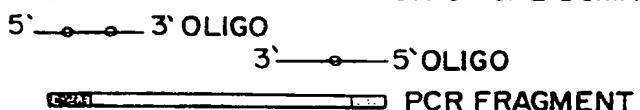


Fig. 10B

B.- PLASMID DNA LINEARIZED INSIDE CH2 DOMAIN AND CO-
TRANSFORMED WITH PCR FRAGMENT INTO COMPETENT DH5 α

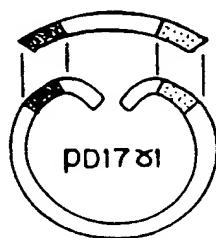
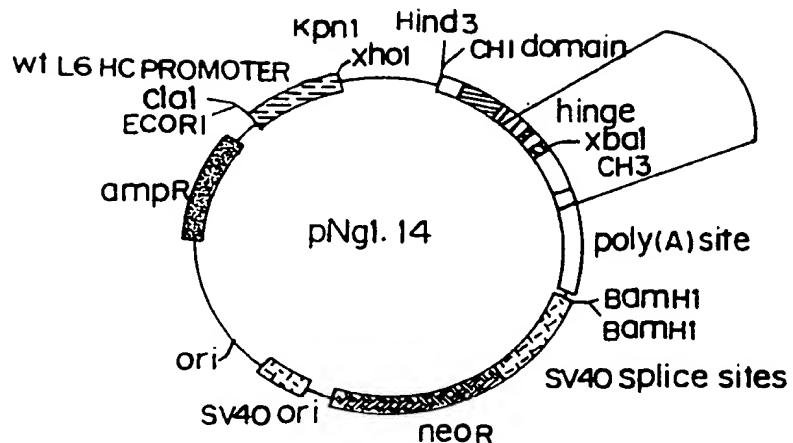
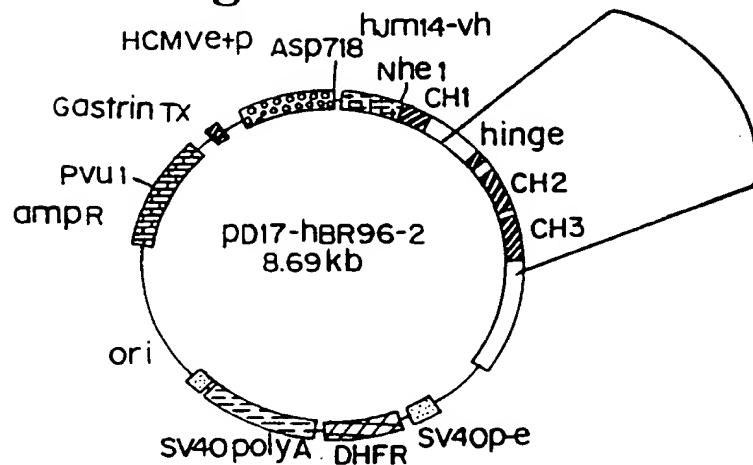
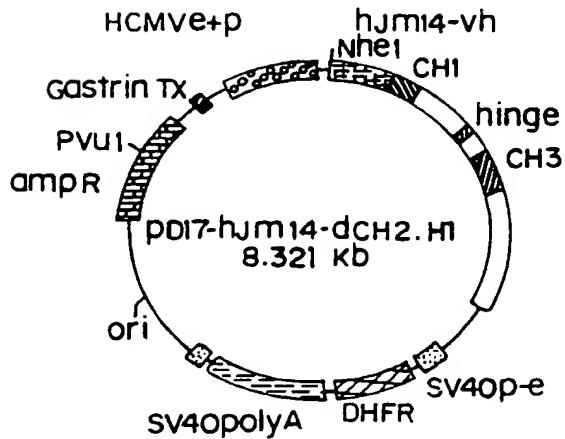


Fig. 10C

C.- CLONING MEDIATED BY HOMOLOGOUS RÉCOMBINATION YIELDS
TRANSFORMANTS HARBOURING RECOMBINANT PLASMIDS.



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Fig. 11**Fig. 12****Fig. 13**

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pD17-cJ-dCH2.H1

FIG. 14A

10	20	30	40	50	60	70	80	90
GACGGATCGG	GAGATCTGCT	AGGTGACTGT	AGGGCGCCG	GCTTCGAATA	GCAGAGTAA	CCTTTTTTT	TAATTTTT	TTATTTTT
CTGCCCTAGCC	CTCTAGACGA	TCCACTGAC	TCCGGGGCC	CGAAGCTTAT	CGGTCTCAT	GGAAAAAAA	ATTAATAAA	AATAAATAA
100	110	120	130	140	150	160	170	180
TTTGAGATGG	AGTTTGGCGC	CGATCTCCG	ATCCCCTATG	GTGCACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTA	AGCCAGTATC
AAACTCTACC	TCAAACCGCG	GCTAGAGGGC	TAGGGATAAC	CAGCTGAGAG	TCATGTTAGA	CGAGACTACG	GGGTATCAAT	TCGGTCATAG
190	200	210	220	230	240	250	260	270
TGCTCCCTGC	TTGTGTGTTG	GAGGTGCGTG	AGTAGTGGCGC	GAGCAAAATT	TAAGCTACAA	CAAGGCAAGG	CTTGACCGAC	AATTGCAATGA
ACGAGGGACG	AACACACAAAC	CTCCAGCGAC	TCATCACGGG	CTCGTTTAA	ATTCGATGTT	GTTCCGTTCC	GAACCTGGCTG	TTAACGTTACT
280	290	300	310	320	330	340	350	360
AGAATCTGCT	TAGGTTAGG	CGTTTGCGC	TGCTTCGCGA	TGTACGGCC	AGATATAACG	GTTGACATTG	ATTATTGACT	AGTTATTAAAT
TCTTAGACGA	ATCCAATCC	GCAAACGGC	ACGAAGGGCT	ACATGCCCGG	TCTATATGCG	CAACTGTAAC	TAATAACTGA	TCAATAAATTA
370	380	390	400	410	420	430	440	450
AGTAATCAAT	TACGGGGTCA	TTAGTTCTATA	GCCCCATATAT	GGAGTTCCGC	GTACATATAAC	TTACGGTAA	TGGCCCGCT	GGCTGACCCG
TCATTAGTTA	ATGCCCTAGT	AATCAAGTAT	CGGGTATATA	CCTCAAGGGC	CAATGTATTG	AATGCATT	ACCGGGCGA	CCGACTGGCG
460	470	480	490	500	510	520	530	540
CCAACGACCC	CCGCCCATGG	ACGTCAATAA	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TGACGTCAA	TGGGGACT
GTTGCTGGG	GGCGGTAAAC	TGGCAGTAC	ATCAAGTGT	TCATATGCCA	AGTACGCC	CTATTGACGT	CAATGACGGT	AAATGGCCCG
550	560	570	580	590	600	610	620	630
ATTACGGTA	AACTGCCAC	TTGGCAGTAC	ATCAAGTGT	TCATATGCCA	AGGGTATCAT	TGCGGTTATC	CTGTAAAGGT	AACTGCGAGTT
TAAATGCCAT	TTGACGGGTG	AACCGTCATG	TAGTTCACAT	AGTATACGGT	TCATGCCGG	GATAACTGCA	GTAACTGCA	TTTACCGGGC
640	650	660	670	680	690	700	710	720
CCTGGCATTA	TGCCCACTAC	ATGACCTAT	GGGACTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTAC	ATGGTGATGC
GGACCGTAT	ACGGGTATG	TACTGGATAA	CCCTGAAAGG	ATGAACCGTC	ATGTAGATGC	ATAATCAGTA	GGGATAATGG	TACCACTACG
730	740	750	760	770	780	790	800	810
GGTTTGGCA	GTACATCAAT	GGGGCGTGGAT	AGGGGTTGA	CTCACGGGGA	TTCACCCCAT	TGACGTCAA	GGGAGTTGT	
CCAAAACCGT	CATGTAGTTA	CCCGCACTA	TGCCCCAACT	GAGTGCCT	AAAGGTTAGA	AGGTGGGTA	ACTGCAGTTA	CCCTCAAAACA
820	830	840	850	860	870	880	890	900
TTTGGCACCA	AAATCAACGG	GACTTTCAA	AATGTCGTAA	CAACTCGCC	CCATTGACGC	AAATGGGGGG	TAGGGCGGT	CGGTGGGAGG
AAACCCGTGT	TTTGTGTGCC	CTGAAAGGT	TTACAGGATT	GTTGAGGGCG	GGTAAACTGCG	TTTACCGGCC	ATCCGGCACAT	GCCACCCCTCC

FIG. 14B

PD17-CJ-dCH2.H1

910	920	930	940	950	960	970	980	990
TCTATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	TTATCGAAA	TCACATAGG	GAGACCCAAG	
AGATATTC	GTCAGAG	ACCGATTGAT	CTCTTGGGTG	ACGAATGACC	GAATAGCTT	AATTATGCTG	AGTGATATCC	CTCTGGGTT
1000	1010	1020	1030	1040	1050	1060	1070	1080
CTTGGTACCA	ATTAAATTG	ATATCTCCT	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGGGCC	GCTGCTAGC
GAACCATGGT	TAATTTAAC	TATAGAGGAA	TCCAGAGCTC	AGAGATCTAT	TGGCCAGTA	GCTAACCTTA	AGAACGCCGG	CGAACGATCG
1090	1100	1110	1120	1130	1140	1150	1160	1170
CACCATGGAG	TTGTGGTTAA	GCTTGGGCC	TCCCTGTCT	TGTTTAAAAA	GGTGTCCAGT	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGAG
GTGGTACCTC	AAACCCAATT	CGAACCGAGA	AGGAACAGGA	ACAAAATTTC	CCACAGGTCA	CACTCACTT	AGACCACTC	AGACCCCTC
1180	1190	1200	1210	1220	1230	1240	1250	1260
GCTTAGTGCA	GCCCTGGAGGG	TCCCTGAAAG	TCTCCTGTGT	AACCTCTGGA	TTCACCTTC	GTGACTATA	CATGTTATGG	GTTCGCCAGA
CGAATCACGT	CGGACCTCCC	AGGGACCTTC	AGGAGACACA	TTGGAGACCT	AAGTGAAAGT	CACTGATAAT	GTACATAACC	CAAGGGTCT
1270	1280	1290	1300	1310	1320	1330	1340	1350
CTCCAGAGAA	GAGGCTGGAG	TGGGTGGCAT	ACATTAGTCA	AGGTGGTGT	ATAACCGACT	ATCCAGACAC	TGTAAGGGT	CGATTCAACCA
GAGGTCTCTT	CTCCGACCTC	ACCCAGGTAA	TGTAATCAGT	TCCACCACTA	TATTGGCTGA	TAGGTCTGTG	ACATTTCCTA	GCTTAAGTGGT
1360	1370	1380	1390	1400	1410	1420	1430	1440
TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	ACCTGCAAAT	GAGCCGTCTG	AAAGTCGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC
AGAGGTCTCT	GTACGGTTTC	TTGTGGGACA	TGGACGTTA	CTCGGCAGAC	TTCAGACTTC	TGTGTGGTA	CATAATGACA	CGTTCTCCGG
1450	1460	1470	1480	1490	1500	1510	1520	1530
TGGACGAGG	GGCCTGGTTT	GCTTACTGGG	GCCAAAGGGAC	TCTGGTCACG	GTCTCTGTAG	CTAGCACAA	GGGGCCCATCG	GTCTTCCCC
ACCTGCTGCC	CGGGACAAA	CGAATGACCC	CGTTCCCTG	AGACCAAGTGC	CAGAGACATC	GATGTTGGTT	CCCCGGTAC	CAGAAGGGGG
1540	1550	1560	1570	1580	1590	1600	1610	1620
TGGCACCTC	CTCCAAGAGC	ACCTCTGGG	GCACAGGGC	CCTGGGCTGC	CTGGTCAGG	ACTACTTCCC	CGAACCCGGTG	ACGGTGTCTG
ACCGTGGAG	GAGGGACTGG	TGGAGACCCC	CGTGTGGCG	GGACCGGACG	GAACAGTTCC	TGATGAAGGG	GCTTGGCAC	TGCCACAGCA
1630	1640	1650	1660	1670	1680	1690	1700	1710
GGAACTCAGG	CGCCCTGACC	AGGGCGGTGC	ACACCTTCCC	GGCTGTCTA	CAGTCCTCAG	GACTCTACTC	CCTCAGGAGC	GTGGTCACCG
CCTTGAGTCC	GCGGGACTGG	TGGCCGCACG	TGTGGAAAGGG	CCGACAGGAT	GTCAAGGAGTC	CTGAGATGAG	GGAGTGTGCG	CACCACTGCG
1720	1730	1740	1750	1760	1770	1780	1790	1800
TGCCCTCAG	CAGGCTGGGC	ACCCAGACCT	ACATCTGCCA	CGTGAATCAC	AAGGCCAGCA	ACACCAAGGT	GGACAGAGAA	GTGGTGTGAGA
ACGGGAGTC	GTGCAACCCG	TGGGTCTGGA	TGTTAGACGTT	TTCAGTGTG	TGTGGTTCTT	TGACTTAGTGT	CCGGTCTTCA	CAACCACTCT

FIG. 14C

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1810	1820	1830	1840	1850	1860	1870	1880	1890
GGCCAGCAC	GGGAGGGAGG	GTTGTCGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGAGG	CATCCGGGT	ATGCCAGCCC	AGTCCAGGGC
CCGGTCGTGT	CCCTCCCTCC	CACAGACGAC	CTTCGGTCCG	AGTCGGGAGG	ACGGACCTGC	GTAGGGCCGA	TACGTCGGGG	TCAGGCCCCG
1900	1910	1920	1930	1940	1950	1960	1970	1980
AGCAAGGCAG	GCCCCGCTG	CCTCTCACCC	CGGAGGCCTC	TGCCGCCCTC	ACTCATGCTC	AGGGAGGGG	TCTTCTGGCT	TTTCCCCAG
TGTTCCCGTC	CGGGGAGAC	GGAGAAGTGG	GCCTCCGGAG	ACGGGGGGGG	TGAGTACGAG	TCCCTCTCCC	AGAAAGACGA	AAAAGGGGTC
1990	2000	2010	2020	2030	2040	2050	2060	2070
GCTCTGGGCA	GGCACAGGCT	AGGTGCCCCCT	AACCCAGGCC	CTGCAACCAA	AGGGGAGGT	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC
CGAGACCCGT	CCGTGTCGCA	TCCACGGGA	TTGGGTCGGG	GACGTGTGTT	TCCCCGTCCA	CGACCCGAGT	CTGGACGGTT	CTCGGTATAG
2080	2090	2100	2110	2120	2130	2140	2150	2160
CGGGAGGACC	CTGGCCCTGA	CTTAAGGCCA	CCCCAAAGGC	CAAACCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCTC	CCAGATTCCA
GGCCCTCCTGG	GACGGGGACT	GGATTCTGGGT	GGGGTTTCCG	GTGTTGAGGG	TGAGGGAGTC	GAGGCTGTGG	AAAGAGGAGG	GGTCTAAGGT
2170	2180	2190	2200	2210	2220	2230	2240	2250
GTAACTCCA	ATCTTCTCTC	TGGAGGCC	AAATCTTGTG	ACAAAACTCA	CACATGCCCA	CCGTGCCAG	GTAAGCCAGC	CCAGGCCCTCG
CATTGAGGGT	TAGAAGAGAG	ACGTCTGGGG	TTTAGAACAC	TGTTTGAGT	GTGTACGGGT	GGCACGGTC	CATTGGTCG	GGTCCGGAGC
2260	2270	2280	2290	2300	2310	2320	2330	2340
CCCTCCAGCT	CAAGGGGGAA	CAGGGCCCT	AGAGTAGCCT	GCATCCAGGG	ACACACCACG	TGGGTACCAA	CATGGTCCGGA	GCCACATGGG
GGGAGGTCGA	GTTCGGCCCT	GTCCACGGGA	TCTCATCGGA	CGTAGTCTCCC	TGTGTGGTGC	ACCCATGGTT	GTACAGGCT	CGGTGTACCT
2350	2360	2370	2380	2390	2400	2410	2420	2430
CAGAGGCCGG	CTCGGCCAC	CCTCTGGCCT	GAGAGTGAACC	GCTGTACCAA	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA
GTCTCCGGCC	GAAGGGGGTG	GGAGACGGGA	CTCTCACTGG	CGACATGGTT	GGAGACAGGG	ATGTCCCCTC	GGGGCTCTTG	GTGTCCACAT
2440	2450	2460	2470	2480	2490	2500	2510	2520
CACCTGCC	CCATCCGGG	ATGAGCTGAC	CAAGAACAG	GTCAGGCTGA	CCTGCCTGCT	CAAAGGCTC	TATCCCAGGC	ACATCGCCGT
GTGGGACGGG	GGTAGGGCCC	TACTCGACTG	GTTCCTGGTC	CACTGGACT	GEACGGACCA	GTTCGGAAG	ATAGGGTGC	TGTAGGGCCA
2530	2540	2550	2560	2570	2580	2590	2600	2610
GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CACTACAAG	ACCAAGCCTC	CCGTGCTGGA	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA
CCTCACCCCTC	TCGTTACCG	TCGGCCTCTT	GTGATGTT	TGGTGGGAG	GGCACGACCT	GAGGCTGCCG	AGGAAGAAGG	AGATGTCGTT
2620	2630	2640	2650	2660	2670	2680	2690	2700
GCTCACCGTG	GACAAGAGCA	GGGGAACTGCA	GGGGAACTGCA	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	ACACGGAGAA	
CGAGTGGCAC	CTGTTCTCGT	CCACCGTCTG	CCACCTGAG	AAGAGTACGA	GGCACTACGT	ACTCCGAGAC	GTGTTGGTGA	TGTGCGTCT

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FIG. 14D

2710	2720	2730	2740	2750	2760	2770	2780	2790
GAGCCTCTCC	CTGTCTCGG	GTAATGAGT	GCGACGGCCG	GCAAGCCCC	GCTCCCCGGG	CTCTCGGGT	CGCACGAGGA	TGCTTGGCAC
CTCGGAGGG	GACAGAGGG	CATTACTCA	CGTGTGGGC	CGTGGGGGG	GAGGGGCC	GAGAGGCCA	GGGTGCTCT	ACGAACCGTG
2800	2810	2820	2830	2840	2850	2860	2870	2880
GTACCCCTG	TACATACTTC	CGGGCGCCC	AGCATGGAAA	TAAGGACCC	AGCGCTGCC	TGGGCCCC	CGAGACTGTG	ATGGTTCTTT
CATGGGGAC	ATGTATGAAG	GGCCCGGGGG	TGGTACCTTT	ATTCGGGG	TGGGACGGG	ACCCGGGAC	GCTCTGACAC	TACCAAGAAA
2890	2900	2910	2920	2930	2940	2950	2960	2970
CCACGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	GGGGCCCAC	GTCcccACAC	TGGCCAGGGC	TGTGCAGGTG
GGTGGCCAGT	CGGGCTCAGA	CTCCGGAACTC	ACCGTACTCC	CTCCGTCTCG	CCAGGGTGA	CAGGGTGTG	ACCGGTCCCG	ACACGTCCAC
2980	2990	3000	3010	3020	3030	3040	3050	3060
TGCCTGGCC	CCCTAGGGTG	GGGCTCAGCC	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT
ACGGACCCGG	GGGATCCAC	CCCAGTCTGG	TCCCCGACGG	GAGCCGTTCC	ACCCCTAA	CGGTGGCACC	GGGAGGGAGG	TGTCGTGGAA
3070	3080	3090	3100	3110	3120	3130	3140	3150
GCCCTGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCCTGGG	GACAGACACA	CAGAGACACA	CTCTGTAGGA	GACTGTCCCTG	TTCTGTGAGC
CGGGACCCGA	CCCCGTGCC	TTCGGGATCC	TCGGGGACCC	CTGTCGTGT	GTTCGGGACG	GAGACATCCT	CTGACAGGAC	AAGACACTCG
3160	3170	3180	3190	3200	3210	3220	3230	3240
GCCCCTGTCC	TCCCCACCTC	CATGCCCACT	CGGGGGCATG	CCTAGTCCAT	GTGGGTAGGG	ACAGGCCCTC	CCTCACCCAT	CTACCCCCAC
CGGGGACAGG	AGGGCTGGAG	GTACGGGTGA	GCCCCCGTAC	GGATCAGGTA	CACGCATCCC	TGTCCGGAG	GGAGTGGGTA	GATGGGGTGA
3250	3260	3270	3280	3290	3300	3310	3320	3330
GGCACTAAC	CCTGGCTGCC	CTGCCCAAGCC	TGGCACCCGC	ATGGGACAC	AACCGACTCC	GGGAACATGC	ACTCTCGGGC	CCTGTGGAGG
CCGTGATTGG	GGACCGACGG	GACGGTCTGG	AGCGTGGGGC	TACCCCTGTG	TTGGCTGAGG	CCCCTGTACG	TGAGGCCCG	GGACACCTCC
3340	3350	3360	3370	3380	3390	3400	3410	3420
GACTGGTCA	GATGCCACACA	CACACACTCA	GCCAGACCC	GTTCACAAA	CCCCGCACTG	AGGTTGGCC	GCCACACGGC	CACCAACAC
CTGACCACTG	CTACGGGTGT	GTGTGTGAGT	CGGGTCTGGG	CAAGTTGTTT	GGGGCGTGTAC	TCCAAACGGG	CGGTGTGCCG	GTGGTGTGTG
3430	3440	3450	3460	3470	3480	3490	3500	3510
ACACGTGCA	GCCTCACACA	CGGAGCTCA	CCCGGGCGAA	CTGCACAGCA	CCCAGACAG	AGCAAGGTCC	TGCGCACAGT	GAACACTCCT
TGTGCACGTG	CGGAGTGTGT	GCCTCGGGACT	GGGGCCCGCTT	GACGTGTGT	GGGTCTGGTC	TCGTTCCAGG	AGCGTGTGCA	CTTGTGAGGA
3520	3530	3540	3550	3560	3570	3580	3590	3600
CGGACACAGG	CCCCCACGAG	CCCCACGGGG	CACCTCAAGG	CCCAAGGCC	TCTGGCGAGC	TTCTCACAT	GCTGACCTGC	TCAGACAAAC
GCCTGTGTCC	GGGGGTGCTC	GGGGTGGCC	GTGGAGTTCC	GGGTGGCTGG	AGAGCCGTG	AAGAGTGTG	CGACTGGAGC	AGTCTGTGTTG

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FIG. 14E

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3610	3620	3630	3640	3650	3660	3670	3680	3690
CGAGCCCTTC	TCTCACAAAGG	GTGCCCTGC	AGCCGCCACA	CACACAGG	GGATCACACA	CCACGTCACG	TCCCTGGCC	TGGCCCACTT
GGTCGGGG	AGAGTGTCC	CACGGGACG	TCGGGGGTGT	GTGTGTGTCC	CCTAGTGTGT	GGTGAAGTGC	AGGGACGGG	ACGGGTGAA
3700	3710	3720	3730	3740	3750	3760	3770	3780
CCCAGTGC	CCCTCCCTG	CAGGACGGAT	CAGGCCCTGAC	TGTGCCCTCT	AGTTGCCAGC	CATCTGTGT	TTGCCCTCC	CCCGTGCCT
GGGTACGGC	GGGAAGGGAC	GTCCTGCTA	GTGGAGCTG	ACACGGAAAGA	TCACGGGTG	GTAAACGATG	AACGGGGAGG	GGGCACGGAA
3790	3800	3810	3820	3830	3840	3850	3860	3870
CCTTGACCT	GGAAAGGTGCC	ACTCCCCACTG	TCCTTICCTA	ATAAAATGAG	GAATTGCAAT	CGCATGTC	GAGTAGGTGT	CATTCTATTG
GGAAACTGGG	CCITCCACGG	TGAGGGTGC	AGGAAAGGAT	TATTTACTC	CTTTAACGTA	GGTAACAGA	CTCATCCACA	GTAAGATAAG
3880	3890	3900	3910	3920	3930	3940	3950	3960
TGGGGGGTGG	GGTGGGGCAG	GACGCAAGG	GGGAGGATTG	GGAAAGCAAT	AGCAGGCCAT	CTGGGGATGC	GGTGGGGCTCT	ATGGGTTCTG
ACCCCCCACC	CCACCCCGTC	CTGTCGTTCC	CCCTCCTAAC	CCTCTGGTA	TGTCGGTAC	GACCCCTACG	CCACCCGAGA	TACCGAAGAC
3970	3980	3990	4000	4010	4020	4030	4040	4050
AGGGGAAAG	AACCAGCTGG	GGCTCTAGG	GGTATCCCCA	CGGCCCTGT	AGGGGGCAT	TAAGGGGGC	GGGTGGGGTG	GTAAAGGGCA
TCCGCCTTTC	TGGTCGACC	CCGAGATCCC	CCATAGGGGT	GGGGGGAC	TGTCGGCGTA	ATTTCGGCCG	CCCACACAC	CAATGGCGGT
4060	4070	4080	4090	4100	4110	4120	4130	4140
CGGTGACCGC	TACACTTGCC	AGGCCCTAG	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCCT	TTCTGCCCCAC	GTTCGCCAC	CCTCTCAAAA
CGCACTGGCG	ATGTGAACGG	TGCGGGATC	GGGGCGAGG	AAAGGAAAG	AAAGGAAAGGA	AAGAGGGGTG	CAAGGGGCC	GGAGAGTTT
4150	4160	4170	4180	4190	4200	4210	4220	4230
AGGGGAAAA	AGGATGGAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	CTAACCTCCGC	CCATCCCGCC	CCTAACTCCG	CCCAGTTCCG
TTCCTTTT	TTCGTACGTA	GAGTTAAC	GTGTTGGTA	TCAGGGGG	GATTGAGGG	GGTAGGGCG	GGATTGAGGC	GGTCAAGGC
4240	4250	4260	4270	4280	4290	4300	4310	4320
CCCATTCTCC	CCCCCATGGC	TGACTAATT	TTTTATTAA	TGCAGAGGCC	GAGGCCGCC	CGGCCCTCTGA	GCTATTCCAG	AAGTAGTGAG
GGGTAAGGG	CGGGTACCG	ACTGATTAA	AAAAATAAAAT	ACGTCTCCGG	CTCCGGGGGA	GCCGGAGACT	CGATAAGGT	TTCATCACTC
4330	4340	4350	4360	4370	4380	4390	4400	4410
GAGGCTTTT	TGGAGGGCTA	GGCTTTGCA	AAAAGCTGG	ACAGCTCAGG	GCTGCGATT	CGCGCAAAC	TTGACGGCAA	TCCTAGCGTG
CTCCGAAAAA	ACCTCCGGAT	CGGAAAACGT	TTTTCGAACC	TGTCGAGTCC	CGACGCTAA	GCGCGTTTG	AAC TGCCGTT	AGGATCGCAC
4420	4430	4440	4450	4460	4470	4480	4490	4500
AAGGCTGGTA	GGATTTATC	CCCGCTGCCA	TCATGGTTCG	ACCATTGAAC	TGCATCGTC	CCGTGTCCCA	AAATATGGGG	ATTGGCAAGA
TTCGGACCAT	CCTAAATAAG	GGGGGACGGT	AGTACCAAGC	TGGTAACCTTG	ACGTAGCAGC	GGCACAGGGT	TTTATAACCC	TAACCGTTCT

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FIG. 14F

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4510	4520	4530	4540	4550	4560	4570	4580	4590
ACGGAGACT	ACCCGGCTT	CCGCTCGGA	ACGAGTCAA	GTACTTCAA	AGAATGCCA	CAACCTCTC	AGTGGAAAGGT	AAACAGAAC
TGGCTCTGGA	TGGGACCGGA	GGCGAGTCT	TGCTCAAGTT	CATGAAGGT	TCTTACTGGT	GTTGGAGAAG	TCACCTTCCA	TTTGTCTTAG
4600	4610	4620	4630	4640	4650	4660	4670	4680
TGGTGATTAT	GGGTAGAAA	ACCTGGTCT	CCATTCTGA	GAAGAACG	CCCTTAAGG	ACAGAATTAA	TATAGTTCTC	AGTAGAGAAC
ACCACTAATA	CCCATCCTT	TGGACCAGA	GGTAAGGACT	CTTCTTAGCT	GGAAATTTC	TGTCTTAATT	ATATCAAGAG	TCATCTCTTG
4690	4700	4710	4720	4730	4740	4750	4760	4770
TCAAAAGAAC	ACCAAGAGGA	GCTCATTT	TTGCCAAAAG	TTGGATGAT	GCCTTAAGAC	TTATTGAACA	ACCGGAATTG	GCAAGTAAG
AGTTCTGG	TGGGCTCT	CGAGTAAGA	AACGGTTTC	AAACCTACTA	CGGAATTCTG	AATAACTTGT	TGGCCTAAC	CGTTCAATTTC
4780	4790	4800	4810	4820	4830	4840	4850	4860
TAGACATGTT	TTGGATAGTC	GGAGGCAGTT	CTGTTACCA	GGAGGCCATG	ATCAACCG	GCCACCTAG	ACTCTTGTG	ACAAGGATCA
ATCTGTACCA	AACCTATCAG	CCTCCGTCAA	GACAATGGT	CCTTCGGTAC	TTAGTTGGTC	CGGTGGAATC	TGAGAAACAC	TGTTCCCTAGT
4870	4880	4890	4900	4910	4920	4930	4940	4950
TGGAGGAATT	TGAAAGTGC	ACGTTTTC	CAGAAATTGA	TTGGGGAAA	TATAAACTTC	TCCCAGAAATA	CCCAGGGTC	CTCTCTGAGG
ACGTCCCTAA	ACTTCACTG	TGCAAAAGGG	GTCTTTAAC	AAACCCCTT	ATATTGAGG	AGGGTCTTAT	GGGTCCCGAG	GAGAGACTCC
4960	4970	4980	4990	5000	5010	5020	5030	5040
TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT	TTGAAGTCTA	CGAGAGAAA	GACTAACAGG	AAGATGCTT	CAAGTTCTCT	GCTCCCCCTC
AGGTCCCTCT	TTTCCGTAG	TTCATATTCA	AACTTCAGAT	GCTCTCTT	CTGATTGTCC	TTCTACGAAA	GTTCAAGAGA	CGAGGGGAGG
5050	5060	5070	5080	5090	5100	5110	5120	5130
TAAGGCTATG	CATTTTATA	AGACCATGGG	ACTTTGCTG	GCTTTAGATC	TCTTTGTGAA	GGAACCTTAC	TTCTGTGGTG	TGACATATT
ATTGATAC	GTAAAATAT	TCTGGTACCC	TGAAAACGAC	CGAAATCTAG	AGAAAACACTT	CCTTGGAAATG	AAGACACCCAC	ACTGTATTAA
5140	5150	5160	5170	5180	5190	5200	5210	5220
GGACAAACTA	CCTACAGAGA	TTTAAAGCTC	TAAGGTAAT	ATAAAATT	TAAGTGTATA	ATGTGTAAA	CTACTGATTC	TAATTGTTTG
CCTGTTGAT	GGATGTCTCT	AAATTGCGAG	ATTCCTATT	TATTTAAAAA	ATTACACATT	TACACAATT	GATGACTAAG	ATTAACAAAC
5230	5240	5250	5260	5270	5280	5290	5300	5310
TGTATTTAG	ATTCACACCT	ATGGAACGTA	TGAATGGGAG	CAGTGGTGG	ATGCCTTAA	TGAGGAAAAC	CTGTTTGTCT	CAGAAGAAAT
ACATAAAATC	TAAGGTTGGA	TACCTGACT	ACTTACCTC	GTCACCCACCT	TACGGAAATT	ACTCCCTTG	GACAAAACGA	GTCTCTTTA
5320	5330	5340	5350	5360	5370	5380	5390	5400
GCCATCTAGT	GATGATGAGG	CTACTGCTGA	CTCTCAACAT	TCTACTCCTC	CAAAAGAGAA	GAGAAAGGTA	GAAGACCCCA	AGGACTTTCC
CGGTAGATCA	CTACTACTCC	GATGAGGACT	GAGAGTTGTA	AGATGAGGAG	GTTTTTCTT	CTCTTCCAT	CTTCTGGGGT	TCCCTGAAAGG

FIG. 14G

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5410	5420	5430	5440	5450	5460	5470	5480	5490
TTCAGAATTG	CTAAGTTTT	TGAGTCAATGC	TGTGTTAGT	AATAAGAACTC	TGCTTGGCTT	TGCTATTAC	ACCAAAAGG	AAAAGCTGC
AAGTCTAAC	GATTCAAAA	ACTCAGTAGC	ACACAATCA	TTATCTTGAG	AACGAACGAA	ACGATAATG	TGGTGTTC	TTTTCGACG
5500	5510	5520	5530	5540	5550	5560	5570	5580
ACTGCTATAC	AAGAAAATTA	TGGAAAATA	TTCTGTAACC	TTTATAAGTA	GGCATAAACAG	TTATAATCAT	AACATACTGT	TTTTCTTAC
TGACGATATG	TTCTTTTAAT	ACCTTTTAT	AGACATTTGG	AAATATTCA	CCGTTATTGTC	AATATTAGTA	TGGTATGACA	AAAAGAAATG
5590	5600	5610	5620	5630	5640	5650	5660	5670
TCCACACAGG	CATAGAGTGT	CTGCTTAA	TAACTATGGT	CAAAATTGT	GTACCTTGTAG	CTTTTAATT	TGAAAGGG	TTAATAAGGA
AGGTGTGCC	GTATCTCAC	GRCGATATT	ATGATACCA	GTTCATRACA	CATGGAAATC	GAABAAATTAA	ACATTTC	AATTATTCCCT
5680	5690	5700	5710	5720	5730	5740	5750	5760
ATATTGATG	TATAGTGCCT	TGACTAGAGA	TCATAATCAG	CCATACCCACA	TTTGTAGAGG	TTTACTTGC	TTTAAAAAAC	CTCCACACC
TATAAACTAC	ATATCACCGA	ACTGATCTCT	AGTATTAGTC	GGTATGGGT	AAACATCTCC	AAATGAAACG	AAATTTTG	GGGGTGTGG
5770	5780	5790	5800	5810	5820	5830	5840	5850
TCCCCCTGAA	CCTIGAACAT	AAAATGAATG	CAATTGTTGT	TGTTAACTTG	TTTATTGGAG	CTTATAATGG	TTACAATAA	AGCAATAGCA
AGGGGGACTT	GGACTTTGTA	TTTTACTTAC	GTAAACAAAC	ACAATGAAAC	AAATAACGTC	GAATATTAC	AATGTTTATT	TCGTTATCGT
5860	5870	5880	5890	5900	5910	5920	5930	5940
TCACAAATT	CACAAATAA	GCATTTTT	CACTGCATT	TAGTTGGGT	TGTTCCRAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG
AGTGTAAA	GTGTTTATT	CGTAAAAAAA	GTGACGTAAG	ATCAAACCCA	AAACAGGTTG	AGTAGTTACA	TAGAATAGTA	CAGACCTAGC
5950	5960	5970	5980	5990	6000	6010	6020	6030
GCTGGATGAT	CCTCCAGGGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCAC	CCCAACTGT	TTATGCAAC	TTATAATGGT	TACAATAAA
CGACCTACTA	GGAGGTGCGG	CCCCTAGAGT	ACGACCTCAA	GAAGGGGTG	GGGTGAAACA	AATAACGTC	AATATTACCA	ATGTTTATT
6040	6050	6060	6070	6080	6090	6100	6110	6120
GCAATAGCAT	ACAAATTTC	ACAAATAAAG	CATTTTTTC	ACTGCAATTCT	AGTTGGGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG
CGTTATCGTA	GTGTTAAAG	TGTTTATTTC	GTAAAAAAAG	TGACGTAAGA	TCACACACAA	ACAGGTTGA	GTAGTTACAT	AGAATAGTAC
6130	6140	6150	6160	6170	6180	6190	6200	6210
TCTGTATACC	GTGGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTCCGT	TGTGAAATTG	TTATCCGCTC	ACAATTCCAC
AGACATATGG	CAGCTGGAGA	TCGATCTCGA	ACCGCATTAG	TACCGATTC	GACAAAGGAC	ACACTTAAC	AATAGGGCAG	TGTTAAGGTG
6220	6230	6240	6250	6260	6270	6280	6290	6300
ACACATACG	AGCCGGAAAGC	ATAAAAGTGT	AAAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	TCACCTGCCG
TGTTGTATGC	TCGGCCCTTCG	TATTCACAT	TTGGGACCCC	ACGGATTACT	CACTCGATTG	AGTGTAAATTA	ACGCAACGGG	AGTGACGGGC

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FIG. 14H

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6310	6320	6330	6340	6350	6360	6370	6380	6390
CTTTCAGTC	GGAAACCTG	TGTCATTAATG	AATCGCCRA	GGCGGGGA	GAGGGGTT	GCGTATTGG	CGCTCTCCG	
AAAAGGTAG	CCCTTGAC	AGCACGGTCG	ACGTAATTAC	TAGCGGGTT	GGCGGCCCT	CTCCGCCAA	CGCATAACCC	GGAGAAGGC
6400	6410	6420	6430	6440	6450	6460	6470	6480
CTTCCTGCT	CACTGACTCG	CTGGCTCGG	TGTTCGGCT	GCGGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG
GAAGGGAGGA	GTGACTGAGC	AGCAAGCCGA	AGCAAGCCGA	CGCCGCTCG	CATAGTCGAG	TGAGTTCCG	CCATTATGCC	AATAGGTGTC
6490	6500	6510	6520	6530	6540	6550	6560	6570
AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC	CGCGTGTCTG	GGTTTTTCC
TTAGTCCCT	ATTGCGTCCT	TTCTTGTACA	CTCGTTTC	GGTCGTTTC	GGTCCCTTG	CATTTCGG	GCGCAACGAC	CGCAAAAGG
6580	6590	6600	6610	6620	6630	6640	6650	6660
ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGGAA	ACCGACAGG	ACTATAAAGA	TACCAAGGC
TATCCGAGGC	GGGGGACTG	CTCGTAGTGT	TTTAGCTGC	GAGTTCAGTC	TCCACCGCTT	TGGGCTGTC	TGATATTCT	ATGGTCCGCCA
6670	6680	6690	6700	6710	6720	6730	6740	6750
TTCCCCCTGG	AAGCTCCCTC	GTGGCTCTC	CTGTTCCGAC	CCTGCGCTT	ACCGGATACC	TGTCGCCCT	TCTCCCTTCG	GGAGGCTGG
AGGGGGGACC	TTCGAGGGAG	CACGGGAG	GCAAGGGCTG	GGACGGCGAA	TGGCCTATGG	ACAGGGGAA	AGAGGGAAAGC	CCTTCGCAAC
6760	6770	6780	6790	6800	6810	6820	6830	6840
CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GTAGGGCTGTT	CGCTCCAAGC	TGGGCTGTC	GCACGAACCC	CCGTTCAAGC
GGAAAAGAGT	TACGAGTGGC	ACATCCATAG	AGTCAAAGCCA	CATCCAGCAA	GCGAGGTTG	ACCCGACACA	CGTGCTTGGG	GGGCAAGTGC
6850	6860	6870	6880	6890	6900	6910	6920	6930
CCGACCCGTG	CCGCTTATCC	GGTAACTATC	GTCTTGACTC	CACCCGGTA	AGACACGGACT	TATGCCACT	GGCAGGAGCC	ACTGGTAACA
GGCTGGGAC	GGGAATAGG	CCATTGATAG	CAGAACTCAG	GTTGGCCAT	TCTGTGTCGA	ATAGGGTGA	CCGTGCTCGG	TGACCATGTT
6940	6950	6960	6970	6980	6990	7000	7010	7020
GGATTAGCAG	AGCGAGGTAT	GTAGGGGTG	CTACAGAGT	CTGGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA
CCTAATCGTC	TGCTCCATA	CATCCGCCAC	GATGTCTCAA	GAACTCACC	ACCGGATTGA	TGCCGATGTG	ATCTCCCTGT	CATAAAACCAT
7030	7040	7050	7060	7070	7080	7090	7100	7110
TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTT
AGACGCGAGA	CGACTTCGGT	CAATGGAAAGC	CTTTTCTCA	ACCATCGAGA	ACTAGGGCGT	TTGTTGGTG	GCGACCATCG	CCACCAAAAG
7120	7130	7140	7150	7160	7170	7180	7190	7200
TTGTTGCAA	GCAGCGAGAA	AAAAAGGATC	TCAAGAGAT	CCTTGTACT	TTCTACGGG	GTCTGACGCT	CAGGGAAACG	
AAACAACGTT	CGTGTCTAA	TGGCGCTCTT	TTTTCCTAG	AGTTCTCTA	GAAGAACTAGA	AAAGATGCC	CAGACTGCGA	GTCAACCTGCG

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FIG. 14I

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7210	7220	7230	7240	7250	7260	7270	7280	7290
AAAACCTACG	TAAAGGGATT	TGGTCATGA	GATTATCAA	AAGGATCTTC	ACCTAGATCC	TTTAATTA	AAAATGAAGT	TTTAATCAA
TTTGAGTGC	AATTCCCTAA	AACCAGTACT	CTAATAGTTT	TTCCTAGAAG	TGGATCTAGG	AAAATTAAT	TTTACTCA	AAATTAGTT
7300	7310	7320	7330	7340	7350	7360	7370	7380
TCTAAAGTAT	ATATGAGTAA	ACTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGGGCCAC	CTATCTAGC	GATCTGTCTA	TTTCGTTCAT
AGATTCATA	TATACTCATT	TGAACCGAC	TGTCAATGGT	TACGAATTAG	TCACTCCGTG	GATAGAGTCG	CTAGACAGAT	AAAGCAAGTA
7390	7400	7410	7420	7430	7440	7450	7460	7470
CCATAGTTGC	CTGACTCCCC	GTTCGTAGA	TAATCTACGAT	ACGGGGGGC	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGGAGAAC
GGGTATCAACG	GACTGAGGGG	CAGCACATCT	ATGTATGCTA	TGCCCTCCCG	ATGGTAGAC	CGGGTCAAG	ACGGTTACTAT	GGCGCTCTGG
7480	7490	7500	7510	7520	7530	7540	7550	7560
CACGCTCACC	GGCTCCAGAT	TTATCAGCAA	TAACACCAGC	AGCCGGAAGG	GCCGAGGCCA	GAAGTGGTCC	TGCCAACTTA	TCCGCCTCCA
GTGCGAGTGG	CCGAGGTCTA	AATAGTCGTT	ATTGGTCTGG	TGGCCTTCC	CGGCTCGGT	CTTCACCCAGG	ACGGTTGAAT	AGGGGGAGGT
7570	7580	7590	7600	7610	7620	7630	7640	7650
TCCAGTCTAT	TAATTGTTGC	CGGGAAAGCTA	GAGTAAGTAG	TTGCCAGTT	AATAGTTTC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG
AGGTCAAGATA	ATTAAACAACG	GCCCTTGAT	CTCATTCATC	AAGGGTCAA	TTATCAAACG	CGTTGAAACA	ACGGTAACGA	TGTCCGTAGC
7660	7670	7680	7690	7700	7710	7720	7730	7740
TGGGTCAAG	CTCGTCGTT	GGTATGGCTT	CATTCAAGTC	CGGTTCCTAA	CGATCAAGGC	GAGTACATG	ATCCCCCATG	TTGTGCAAAA
ACACAGTGC	GAGGAGCAA	CCATACCGAA	GTAAAGTCGAG	GCCAAAGGGT	GCTAGTTTCG	CTCAATGTAC	TAGGGGTAC	AACACGTTT
7750	7760	7770	7780	7790	7800	7810	7820	7830
AAGGGTAG	CTCCTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTGGCC	GCAGTGTAT	CACTCATGGT	TATGGCAGCA	CTGCATAATT
TTGCCAATC	GAGGAAGCCA	GGAGGCTAGC	AAACAGTCTTC	ATTCAACGG	CGTCACAATA	GTGAGTACCA	ATACCGTGT	GACGTATTAA
7840	7850	7860	7870	7880	7890	7900	7910	7920
CTCTTACTGT	CATGCCATCC	GTAAGATGCT	TTCTGTGAC	TGGTGAAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGGGACCGA
GAGAATGACA	GTACGGTAGG	CATTCTACGA	AAAGACACTG	ACCAACTCATG	AGTTGGTTCA	GTAAGACTCT	TATCACATAC	GCCGCTGGCT
7930	7940	7950	7960	7970	7980	7990	8000	8010
GTTGCTCTTG	CCCGGGGTCA	ATACGGGATA	ATACCGGCC	ACATAGCAGA	ACTTTAAAG	TGCTCATCAT	TGGAAAAACGT	TCTTCGGGGC
CAACGAGAAC	GGGGCGCAGT	TATGCCCTAT	TATGGCGGG	TGTATGTCT	TGAATTTTC	ACGGTAGTA	ACCTTTGCA	AAATGAAAGT
8020	8030	8040	8050	8060	8070	8080	8090	8100
GAAGAACTTC	AAAGGATCTTA	CCGCTGGTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAACGTGATC	TTCAGGATCT	TTTACTTTCA
CTTTTGAG	TTCTAGAAT	GGCGACAACT	CTAGGTCAAG	CTACATTGGG	TGAGCACGTG	GGTTGACTAG	AAGTCGTAGA	AAATGAAAGT

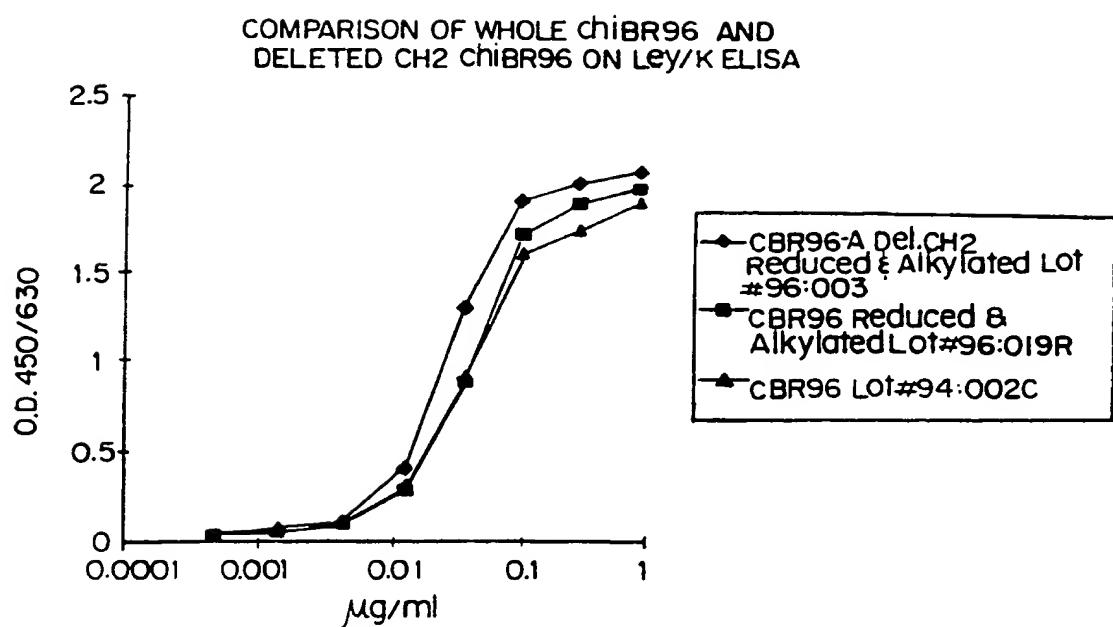
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FIG. 14J

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8110	8120	8130	8140	8150	8160	8170	8180	8190
CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAG	GGATAAGGG	CGACACGGG	ATGTTGAATA	CTCATACTCT
GGTCGCAAAG	ACCCACTCGT	TTTTGTCTT	CCGTTAACG	GGGTTTTTC	CCTTATTCCC	GCTGTGCCCT	TACAACCTAT	GAGTATGAGA
8200	8210	8220	8230	8240	8250	8260	8270	8280
TCCTTTCA	ATATTATTGA	AGCATTATC	AGGTTATTG	TCTCATGAGC	GGATAACATAT	TTGAATGTAT	TTAGAAAAT	AAACAAATAG
AGGAAAAAGT	TATAATAACT	TCGTAATAAC	TCCCAATAAC	AGAGTACTCG	CCTATGTATA	AACTTACATA	ATCTTTTA	TTTGTTTATC
8290	8300	8310	8320	8330				
GGGTTCCGG	CACATTCCC	CGAAAAGTGC	CAACTGACGT	C				
CCCAAGGGCG	GTGTAAAGGG	GCTTTTCACG	GTGGACTGCA	G				

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Fig. 15

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hBR96-2B:L235 to A235 and G237 to A237

hBR96-2C:E318 to S318, K320 to S320, and K322 to S322

hBR96-2D:P331 to A331

hBR96-2E:L235 to A235, G237 to A237, E318 to S318, K320 to S320, and K322 to S322

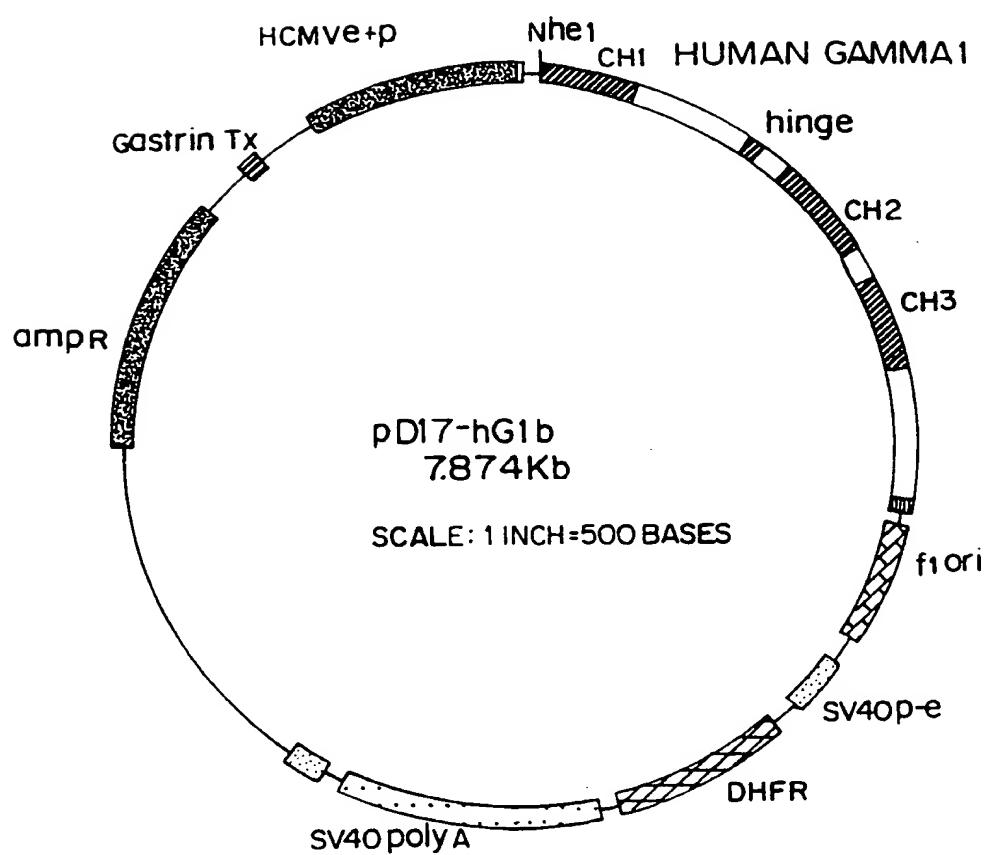
hBR96-2F:L235 to A235, G237 to A237, and P331 to A331

hBR96-2G:E318 to S318, K320 to S320, K322 to S322, and P331 to A331

hBR96-2H: L235 to A235, G237 to A237, E318 to S318, K320 to S320, K322 to S322, and P331 to A331

FIG. 16

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Fig. 17

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FIG. 18A

1	GGTACCAATT	TAAATTGATA	TCTCCTTAGG	TCTCGAGTCT	CTAGATAACC
51	GGTCAATCGA	TTGGAATTCT	TGCGGCCGCT	TGCTAGCCAC	CATGGAGTTG
101	TGGTTAACGT	TGGTCTTCCT	TGTCCTTGTT	TTAAAAGGTG	TCCAGTGTGA
151	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	AGTGCAGCCT	GGAGGGTCCC
201	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCACTGA	CTATTACATG
251	TATTGGGTTTC	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT
301	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT
351	TCACCATCTC	CAGAGACAAT	GCAAAGAAC	GCCTGTACCT	GCAAATGAAC
401	AGCCTGAGGG	ACGAGGACAC	AGCCGTGTAT	TACTGTGAA	GAGGCCTGGC
451	GGACGGGCC	TGGTTTGCTT	ACTGGGGCCA	AGGGACTCTG	GTCACGGTCT
501	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	ACCCTCCTCC
551	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA
601	CTTCCCCGAA	CCGGTGACGG	TGTCGTGGAA	CTCAGGCGCC	CTGACCAGCG
651	GCGTGCACAC	CTTCCCGGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC
701	AGCAGCGTGG	TCACCGTGCC	CTCCAGCAGC	TTGGGCACCC	AGACCTACAT
751	CTGCAACGTG	AATCACAAAGC	CCAGCAACAC	CAAGGTGGAC	AAGAAAGTTG
801	GTGAGAGGCC	AGCACAGGGA	GGGAGGGTGT	CTGCTGGAAG	CCAGGCTCAG
851	CGCTCCIGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA
901	AGGCAGGCC	CGTCTGCCTC	TTCACCCGG	GGCCTCTGCC	CGCCCCACTC
951	ATGCTCAGGG	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCA
1001	CAGGCTAGGT	GCCCCTAACC	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG
1051	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	AGGACCCCTGC	CCCTGACCTA
1101	AGCCCACCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	GACACCTTCT
1151	CTCCTCCCAG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCAAAT
1201	CTTGTGACAA	AACTCACACA	TGCCCACCGT	GCCCAGGTAA	GCCAGCCCAG
1251	GCCTCGCCCT	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT
1301	CCAGGGACAG	GCCCCAGCCG	GGTGCTGACA	CGTCCACCTC	CATCTCTTCC

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		235	237		
1351	TCAGCACCTG	AACTCCTGGG	GGGACCGTCA	GTCTTCCTCT	TCCCCCCAAA
1401	ACCCAAGGAC	ACCCTCATGA	TCTCCCGGAC	CCCTGAGGTC	ACATGCGTGG
1451	TGGTGGACGT	GAGCCACGAA	GACCTGAGG	TCAAGTTCAA	CTGGTACGTG
1501	GACGGCGTGG	AGGTGCATAA	TGCCAAGACA	AAGCCGCGGG	AGGAGCAGTA
1551	CAACAGCACG	TACCGTGTGG	TCAGCGTCCT	CACCGTCCTG	CACCAAGGACT
		318	320	322	
1601	GGCTGAATGG	CAAGGAGTAC	AAGTGCAAGG	TCTCCAACAA	AGCCCTCCCA
		331			
1651	GCCCCCATCG	AGAAAACCAT	CTCCAAAGCC	AAAGGTGGGA	CCC GTGGGGT
1701	GCGAGGGCCA	CATGGACAGA	GGCCGGCTCG	GCCCACCCCTC	TGCCCTGAGA
1751	GTGACCGCTG	TACCAACCTC	TGTCCCTACA	GGGCAGCCCC	GAGAACACAA
1801	GGTGTACACC	CTGCCCCCAT	CCC GGATGA	GCTGACCAAG	AACCAGGTCA
1851	GCCTGACCTG	CCTGGTCAAA	GGCTTCTATC	CCAGCGACAT	CGCC GTGGAG
1901	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAAC	TACAAGACCA	CGCCTCCCGT
1951	GCTGGACTCC	GACGGCTCCT	TCTTCCCTCTA	CAGCAAGCTC	ACCGTGGACA
2001	AGAGCAGGTG	GCAGCAGGGG	AACGTCTTCT	CATGCTCCGT	GATGCATGAG
2051	GCTCTGCACA	ACCACTACAC	GCAGAACAGC	CTCTCCCTGT	CTCCGGTAA
2101	ATGAGTGCAG	CGGCCGGCAA	GCCCCCGCTC	CCC GGCTCT	CGCGGTGCGA
2151	CGAGGGATGCT	TGGCACGTAC	CCC CTGTACA	TACTTCCCGG	GCGCCCAGCA
2201	TGGAAATAAA	GCACCCAGCG	CTGCCCTGGG	CCC CTGCGAG	ACTGTGATGG
2251	TTCTTTCCAC	GGGTCAAGGCC	GAGTCTGAGG	CCTGAGTGGC	ATGAGGGAGG
2301	CAGAGCGGGT	CCC ACTGTCC	CCACACTGGC	CCAGGCTGTG	CAGGTGTGCC
2351	TGGGCCCCCT	AGGGTGGGGC	TCAGCCAGGG	GCTGCCCTCG	GCAGGGTGGG
2401	GGATTTGCCA	GGCGTGGCCCT	CCCTCCAGCA	GCACCTGCC	TGGGCTGGGC
2451	CACGGGAAGC	CCTAGGAGCC	CCTGGGGACA	GACACACAGC	CCCTGCCTCT
2501	GTAGGAGACT	GTCCTGTTCT	GTGAGCGCCC	CTGTCCCTCCC	GACCTCCATG
2551	CCCACTCGGG	GGCATGCCTA	GTCCATGTGC	GTAGGGACAG	GCCCTCCCTC
2601	ACCCATCTAC	CCCCACGGCA	CTAACCCCTG	GCTGCCCTGC	CCAGCCTCGC
2651	ACCCGCATGG	GGACACAAACC	GAATCCGGGG	ACATGCACTC	TCGGGCCCTG
2701	TGGAGGGACT	GGTGCAGATG	CCCACACACA	CACTCAGCCC	AGACCCGTTTC
2751	AACAAACCCC	GCACTGAGGT	TGGCCGGCCA	CACGGCCACC	ACACACACAC
2801	GTGCACGCCT	CACACACGGA	GCCTCACCCG	GGCGAAGTGC	ACAGCACCCA

FIG. 18B

SUBSTITUTE SHEET (RULE 26)

2851	GACCAGAGCA	AGGTCCCTCGC	ACACGTGAAC	ACTCCTCGGA	CACAGGCC
2901	CACGAGCCCC	ACCGGGCACC	TCAAGGCCA	CGAGCCTCTC	GGCAGCTTCT
2951	CCACATGCTG	ACCTGCTCAG	AATAACCCAG	CCCTCCTCTC	ACAAGGGTGC
3001	CCCTGCAGCC	GCCACACACA	CAGGGGAT	CACACACCAC	GTCACGTCCC
3051	TGGCCCTGGC	CCACTTCCCA	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAGC
3101	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	TGTTGTTTGC	CCCTCCCCCG
3151	TGCCTTCCTT	GACCCTGGAA	GGTGCCACTC	CCACTGTCT	TTCCTAATAA
3201	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	CTATTCTGGG
3251	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGA	GGATTGGAA	GACAATAGCA
3301	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAAC
3351	AGCTGGGCT	CTAGGGGTA	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAAG
3401	CGCGCGGGT	GTGGTGGTTA	CGCGCAGCGT	GACCGCTACA	CTTGCCAGCG
3451	CCCTAGCGCC	CGCTCCCTTC	GCTTCTTCC	CTTCCTTCT	CGCCACGTTC
3501	GCCGGGCCTC	TCAAAAAGG	GAAAAAAAGC	ATGCATCTCA	ATTAGTCAGC
3551	AACCATAGTC	CCGCCCCCTAA	CTCCGCCCCAT	CCCGCCCCCTA	ACTCCGCCA
3601	GTTCCGCCA	TTCTCCGCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA
3651	GAGGCCGAGG	CCGCCTCGGC	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG
3701	CTTTTTTGGA	GGCCTAGGCT	TTTGCAAAA	GCTTGGACAG	CTCAGGGCTG
3751	CGATTTCGCG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	CTGGTAGGAT
3801	TTTATCCCCG	CTGCCATCAT	GGTCGACCA	TTGAACTGCA	TCGTCGCCGT
3851	GTCCCCAAAT	ATGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC
3901	TCAGGAACGA	GTTCAAGTAC	TTCCAAGAA	TGACCACAAC	CTCTTCAGTG
3951	GAAGGTAAAC	AGAATCTGGT	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT
4001	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	AATTAATATA	GTTCAGTA
4051	GAGAACTCAA	AGAACCAACCA	CGAGGGAGCTC	ATTTTCTTGC	CAAAGTTTG
4101	GATGATGCCT	TAAGACTTAT	TGAACAAACCG	GAATTGGCAA	GTAAAGTAGA
4151	CATGGTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAAGGAA	GCCATGAATC
4201	AACCAGGCCA	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTGAA
4251	AGTGACACGT	TTTTCCCAGA	AATTGATTG	GGGAAATATA	AACTTCTCCC
4301	AGAATACCCA	GGCGTCCTCT	CTGAGGTCCA	GGAGGAAAAA	GGCATCAAGT

FIG. 18C**SUBSTITUTE SHEET (RULE 26)**

4351	ATAAGTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	TGCTTTCAAG
4401	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT
4451	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC
4501	ATAATTGGAC	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA
4551	AATTTTTAAG	TGTATAATGT	GTAAACTAC	TGATTCTAAT	TGTTTGTGTA
4601	TTTAGATTC	CAACCTATGG	AACTGATGAA	TGGGAGCAGT	GGTGGAATGC
4651	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	TCTAGTGATG
4701	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA
4751	AAGGTAGAAG	ACCCCAAGGA	CTTCCCTTCA	GAATTGCTAA	GTTTTTTGAG
4801	TCATGCTGTG	TTTAGTAATA	GAACCTTGC	TTGCTTTGCT	ATTTACACCA
4851	CAAAGGAAAA	AGCTGCACTG	CTATACAAGA	AAATTATGGA	AAAATATTCT
4901	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	AATCATAACA	TACTGTTTT
4951	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	TATGCTCAA
5001	AATTGTGTAC	CTTAGCTTT	TTAATTGTA	AAGGGGTTAA	TAAGGAATAT
5051	TTGATGTATA	GTGCCCTTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTTG
5101	TAGAGGTTTT	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG
5151	AAACATAAAA	TGAATGCAAT	TGTTGTTGTT	AACTTGTAA	TTGCAGCTTA
5201	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	AAATTTCACA	AATAAAGCAT
5251	TTTTTTCACT	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	CAATGTATCT
5301	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT
5351	GGAGTTCTTC	GCCCCACCCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA
5401	AATAAAGCAA	TAGCATCAC	AATTCACAA	ATAAAGCATT	TTTTTCACTG
5451	CATTCTAGTT	GTGGTTTGTC	CAAACTCATC	AATGTATCTT	ATCATGCTG
5501	TATAACCGTCG	ACCTCTAGCT	AGAGCTTGGC	GTAATCATGG	TCATAGCTGT
5551	TTCCCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	CATACGAGCC
5601	GGAAGCATAA	AGTGTAAAGC	CTGGGGTGGC	TAATGAGTGA	GCTAACTCAC
5651	ATTAATTGCG	TTGCGCTCAC	TGCCCCCTTT	CCAGTCGGGA	AACCTGTCGT
5701	GCCAGCTGCA	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCCT
5751	ATTGGGCGCT	CTTCCGCTTC	CTCGCTCACT	GACTCGCTGC	GCTCGGTCGT
5801	TCGGCTGCAG	CGAGCGGTAT	CAGCTCACTC	AAAGGCGGTA	ATACGGTTAT

FIG. 18D
SUBSTITUTE SHEET (RULE 26)

5851	CCACAGAAATC	AGGGGATAAAC	GCAGGAAAGA	ACATGTGAGC	AAAAGGCCAG
5901	CAAAAGGCCA	GGAACCGTAA	AAAGGCCGCG	TTGCTGGCGT	TTTTCCATAG
5951	GCTCCGCCCG	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT
6001	GGCGAAACCC	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC
6051	TCCCTCGTGC	GCTCTCCTGT	TCCGACCCCTG	CCGCTTACCG	GATACCTGTC
6101	CGCCTTCTC	CCTTCGGGAA	GCGTGGCGCT	TTCTCAATGC	TCACGCTGTA
6151	GGTATCTCAG	TCGGGTGTA	GTCGTTGCT	CCAAGCTGGG	CTGTGTGCAC
6201	GAACCCCCCG	TTCAGCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT
6251	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG
6301	GTAACAGGAT	TAGCAGAGCG	AGGTATGTA	GCGGTGCTAC	AGAGTTCTTG
6351	AAGTGGTGGC	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG
6401	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	AAGAGTTGGT	AGCTCTTGAT
6451	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	TTTTTTTGT	TTGCAAGCAG
6501	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC
6551	TACGGGGTCT	GACGCTCACT	GGAACGAAAA	CTCACGTTAA	GGGATTITGG
6601	TCATGAGATT	ATCAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAAAAA
6651	TGAAGTTTA	AATCAATCTA	AAGTATATAT	GAGTAAACTT	GGTCTGACAG
6701	TTACCAATGC	TTAACAGTG	AGGCACCTAT	CTCAGCGATC	TGTCTATTTC
6751	GTTCATCCAT	AGTTGCCTGA	CTCCCCGTG	TGTAGATAAC	TACGATACGG
6801	GAGGGCTTAC	CATCTGGCCC	CAGTGCTGCA	ATGATACCGC	GAGACCCACG
6851	CTCACCGGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG
6901	AGCGCAGAAG	TGGTCCTGCA	ACTTTATCCG	CCTCCATCCA	GTCTATTAAT
6951	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG	CCAGTTAATA	TTTGCACAA
7001	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	GTCACGCTCG	TCGTTGGTA
7051	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	TACATGATCC
7101	CCCATGTTGT	GCAAAAAAAGC	GGTTAGCTCC	TTCGGTCTC	CGATCGTTGT
7151	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC
7201	ATAATTCTCT	TACTGTCATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT
7251	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG
7301	CTCTTGCCCG	GCGTCAATAC	GGGATAATAC	CGCGCCACAT	AGCAGAACTT

FIG. 18E

SUBSTITUTE SHEET (RULE 26)

7351	TAAAAGTGCT	CATCATTGGA	AAACGTTCTT	CGGGGCGAAA	ACTCTCAAGG
7401	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG	TAACCCACTC	GTGCACCCAA
7451	CTGATCTTCA	GCATCTTTA	CTTCACCAG	CGTTTCTGGG	TGAGCAAAAA
7501	CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT
7551	TGAATACTCA	TAATCTTCCT	TTTCAATAT	TATTGAAGCA	TITATCAGGG
7601	TTATTGTCTC	ATGAGCGGAT	ACATATTGTA	ATGTATTTAG	AAAAATAAAC
7651	AAATAGGGT	TCCGCGCACCA	TTTCCCCGAA	AAGTGCACC	TGACGTCGAC
7701	GGATCGGGAG	ATCTGCTAGG	TGACCTGAGG	CGCGCCGGCT	TCGAATAGCC
7751	AGAGTAACCT	TTTTTTTAA	TTTTTATTITA	TTTTTATTTTT	GAGATGGAGT
7801	TTGGCGCCGA	TCTCCCGATC	CCCTATGGTC	GAATCTCAGT	ACAATCTGCT
7851	CTGATGCCGC	ATAGTTAACG	CAGTATCTGC	TCCCTGCTTG	TGTGTTGGAG
7901	GTCGCTGAGT	AGTGCAGCAG	CAAAATTAA	GCTACAACAA	GGCAAGGCTT
7951	GACCGACAAT	TGCATGAAGA	ATCTGCTTAG	GGTTAGGCCT	TTTGCCTGTC
8001	TTCGCGATGT	ACGGGCCAGA	TATA CGCTT	GACATTGATT	ATTGACTAGT
8051	TATTAATAGT	AATCAATTAC	GGGGTCATTA	GTTCATAGCC	CATATATGGA
8101	GTTCCCGCGT	ACATAACTTA	CGGTAAATGG	CCCGCCTGGC	TGACCGCCCA
8151	ACGACCCCCG	CCCATTGACG	TCAATAATGA	CGTATGTTCC	CATAGTAACG
8201	CCAATAGGGA	CTTCCATG	ACGTCAATGG	GTGGACTATT	TACGGTAAAC
8251	TGCCCACTTG	GCAGTACATC	AA GTGTATCA	TATGCCAAGT	ACGCCCCCTA
8301	TTGACGTCAA	TGACGGTAAA	TGGCCCGCCT	GGCATTATGC	CCAGTACATG
8351	ACCTTATGGG	ACTTTCTTAC	TTGGCAGTAC	ATCTACGTAT	TAGTCATCGC
8401	TATTACCATG	GTGATGCGGT	TTTGGCAGTA	CATCAATGGG	CGTGGATAGC
8451	GGTTTGACTC	ACGGGGATTT	CCAAGTCTCC	ACCCCATGTA	CGTCAATGGG
8501	AGTTTGTTTT	GGCACCAAAA	TCAACGGGAC	TTTCCAAAAT	GTCGTAACAA
8551	CTCCGCCCA	TTGACGCAA	TGGCGGTAG	GCGTGTACGG	TGGGAGGTCT
8601	ATATAAGCAG	AGCTCTCTGG	CTAACTAGAG	AACCCACTGC	TTACTGGCTT
8651	ATCGAAATTAA	ATACGACTCA	CTATAGGGAG	ACCCAAAGCTT	

FIG. 18F
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FIG. 19A

		pD17-hG1b			
10	TAATTGATA	20	30	40	50
GGTACCAATT	TTAATTGATA	TCTCCCTTAGG	TCTCGAGTCT	CTAGATAACC	GGTCAAATCGA
CCATGGTTAA	ATTAACTAT	AGAGGAATCC	AGAGGTCAAGA	GATCTTATGG	CCAGTTAGT
70	TGCGGCCGCT	80	90	100	110
TGGAAATTCT	ACGGCCGCGA	TGCTAGCACC	AAGGGCCCAT	CGGTCTTCCC	CCTGGCACCC
AACCTTAAGA	ACGGCCGCGA	ACGATGTTGG	TTCCCCGGTA	GCCAGAAGGG	GGACCCGTGGG
130	GCACCTCTGG	140	150	160	170
TCCCTCAAGA	CGTGGAGAC	GGGCACAGCG	GCCCTGGCT	GCCCTGGTCAA	GGACTACTTC
AGGAGCTCT	CGTGGAGAC	CCCGTGTGCG	CGGGACCCGA	CGAACCCAGTT	CCTGATGAG
190	TGACGGTGT	200	210	220	230
CCCGAACCGG	ACTGCCAACG	GTGGMACTCA	GGGGCCCTGA	CCAGGGGGGT	GCACACCTTC
GGGCTGGCC	ATGTCAGGA	CACCTGAGT	CCGGGGGACT	GTTGCCCCCA	CGTGTTGAAAG
250	TACAGTCCCT	260	270	280	290
CCGGCTGTCC	GGCCGACGG	AGGACTCTAC	TCCCTCAGCA	GGGTGGTCAC	CGTGCCTCC
GGCCGACGG	ATGTCAGGA	TCCTGAGATG	AGGGAGTCGT	CGCACCCAGTG	GCACGGGAGG
310	GOACCCCAAC	320	330	340	350
TGTCGAACC	CGTGGGTCTG	CTACATCTGC	AACGTGAATC	ACAAGCCCAG	CAACACCAAG
AGCAGCTTGG	ATGTCAGGA	GATGTAGACG	TTGCACTTAG	TGTTCGGTTC	GTTCGGTTC
370	AGTGGAAAGA	380	390	400	410
CACCTGTTCT	TTCAACCACT	GAGGCCAGCA	CAGGGGGAAA	GGGTGTCTGC	TGTAAGCCAG
GTGGAAAGA	AGTGTGGTGA	CTCCGGTCGT	GTCCCTCCCT	CCCACAGACG	ACCTTGGTC
430	CCTGCCCTGA	440	450	460	470
GCTCAGGGCT	GGACCGAACCT	CCCATCCCGG	CTATGCCGCC	CCAATCCAGG	GCAGGCAAGG
CGAGTCGGCA	GGAGGAAAGT	GGCTAGGGCC	GATACTGGGG	GGTCAAGGTCC	CETCGTCCG
490	TGCCCTCTCA	500	510	520	530
AGGCCCGTC	TCCGGGGCAG	CCGGGAGGCC	TCTGCCCGCC	CCACTCTATGC	TCAGGGAGAG
TCCGGGGCAG	ACGGAGAAGT	GGGCCCTCCGG	AGACGGGGGG	GGTGGAGTACG	AGTCCTCTC
550	CTTTTCTGG	560	570	580	590
GGTCTCTCTG	CGAAAAGGGG	AGGCTCTGGG	CAGGGCACAGG	CTAGGTCTCC	CTAACCCAGG
CCAGAAAGCC	TCCGAGAC	TCCGAGACCC	GTCCCTGTCC	GATCCACGGG	GATTGGTCC

FIG. 19B**pD17-hG1b**

610	620	630	640	650	660
CCCTGCACAC	AAAGGGCAG	GTGCTGGCT	CAGACCTGCC	AAGGCCATA	TCCGGGAGGA
GGGACGTGTG	TTTCCCCGTC	CACGACCCGA	GTCTGGACGG	TTCTCGGTAT	AGGCCCTCT
670	680	690	700	710	720
CCCTGCCCC	GACCTAAGCC	CACCCAAAG	GCCAAACTCT	CCACTCCCTC	AGCTCGGACA
GGGACGGGA	CTGGATTCGG	GTGGGGTTTC	CGGTTTGAGA	GGTGAGGGAG	TCGAGCCCTGT
730	740	750	760	770	780
CCTTCTCTCC	TCCAGATTG	CAGTAACCTCC	CAATCTTCTC	TCTGCAGAGC	CCAATCTTG
GGAAAGGAGG	AGGGTCTAAG	GTCATTGAGG	GTTAGAAAG	AGACGTCTCG	GGTTTAGAAC
790	800	810	820	830	840
TGACAAACT	CACACATGCC	CACCGTCCC	AGGTAAGCCA	GCCCAGGCCT	CGCCCTCCAG
ACTGTTTGA	GTGGTACGG	GTGGCACGGG	TCCATTGCGT	CGGGTCGGA	GGGGAGGTC
850	860	870	880	890	900
CTCAAGGGG	GACAGGTGCC	CTAGAGTAGC	CTGCATCCAG	GGACAGGCC	CAGCCGGGTG
GAGTTCCGCC	CTGTCCACGG	GATCTCATCG	GACGTAGTC	CCTGTCCGGG	GTCGGCCAC
910	920	930	940	950	960
CTGACACGT	CACCTCCATC	TCTTCCTCAG	CACCTGAAC	CCTGGGGGA	CGGTCAGTCT
GACTGTGCA	GTGGAGGTAG	AGAAGGAGTC	GTGGACTTGA	GGACCCCCCT	GGCAGTCAGA
970	980	990	1000	1010	1020
TCCTCTTCCC	CCCAAAACCC	AAGGACACCC	TCATGATCTC	CGGGACCCCT	GAGGTCACAT
AGGAGAAGGG	GGGTTTGGG	TTCCCTGGGG	AGTACTAGAG	GGCCCTGGGA	CTCCAGTGT
1030	1040	1050	1060	1070	1080
GCGTGGGGT	GGACGTGAGC	CACGAAGACC	CTGAGGTCAA	GTTCAAACTGG	TACGTGGACG
CGCACCCACCA	CCTGCACTCG	GTGCTTCTGG	GACTCCAGTT	CAAGTTGACC	ATGCACCTGC
1090	1100	1110	1120	1130	1140
GCGTGGGGT	GCATAATGCC	AAGACAAAGC	CGGGGGAGGA	GCAGTACAAC	AGCACGTACC
CGCACCCACCA	CGTATTACGG	TTCTGTTCTG	GCGCCCTCCCT	CGTCATGTTG	TCGTGCAATGG
1150	1160	1170	1180	1190	1200
GTGTGGTCAG	CGTCTCTCAC	GTCCTGCACC	AGGACTGGCT	GAATGGCAAG	GAGTACAAGT
CAACCAAGTC	GCAGGAGTGG	CAGGACGTGG	TCCTGACCGA	CTTACCGTTC	CTCATGTTCA

FIG. 19C

pD17-hG1b			
322	1210	1220	1230
GCAAGGTCTC	CAACAAAGCC	CTCCCCAGCCC	CCATCGAGAA
CGTTCCAGAG	GTGTTTCGGG	GAGGGTGGG	GGTAGCTCTT
1270	1280	1290	1300
GTGGGACCCG	TGGGGTGCGA	GGGCCACATG	GACAGAGGCC
CACCCCTGGGC	ACCCCACGCT	CCGGGTGTAC	CTGTCCTCGG
1330	1340	1350	1360
CTGAGAGTGA	CCGCTGTACC	AACCTCTGTC	CCTACAGGGC
GAATCTCACT	GGCGACATGG	TTGGAGACAG	GGATGTCCCC
1390	1400	1410	1420
TACACCCCTGC	CCCACATCCCC	GGATGAGCTG	ACCAAGAACCC
ATGTTGGGACG	GGGGTAGGGC	CCTACTCGAC	TGGTTCTTGG
1450	1460	1470	1480
GTCAGGGCT	TCTATCCCAG	CGACATGCC	GTTGGAGTGGG
CAGTTTCCGA	AGATAGGGTC	GCTGTAGGG	CACCTCACCC
1510	1520	1530	1540
AACAACCTACA	AGACCAACGCC	TCCCGTGCTG	GACTCCGAGC
TTGGTTGATGT	TCTGGTGGGG	AGGGCACGAC	CTGAGGCTGC
1570	1580	1590	1600
AAGCTCACCG	TGGACAAGAG	CAGGTGGAG	CAGGGGAAAG
TTTCGAGTGGC	ACCTGTTCTC	GTCCACCGTC	GTCCCCTGTG
1630	1640	1650	1660
CATGAGGGCTC	TGCACAAACCA	CTACACCGAG	AAGAGCCTCT
GTACTCCGAG	ACGGTGTGGT	GATGTGGTGC	TTCTCGGAGA
1690	1700	1710	1720
GTGGGACGGC	CGGCAAGCCC	CCGGTCCCCG	GGCTCTCGGG
CACGCTGCCG	GCCGGTTCGGG	GGCGAGGGC	CCGAGAGGCC
1750	1760	1770	1780
ACGTACCCC	TGTACATACT	TCCCGGGCGC	CCAGCATGGA
TGCATGGGG	ACATGTTATGA	AGGGCCCGCG	GGTCGTACT

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FIG. 19D

					pD17-hG1b
1810	1820	TGCGAGACTG	TGATGGTCT	TTCCACGGGT	1850
CCTGGCCCC	TGCGAGACTG	ACTACCAAGA	AAGTGGCCA	GAGGCCAGT	1860
GGACCGGGG	ACGCTCTGAC			GTCCGGCTCA	CTGAGGCC
				GAATCGGAC	
1870	1880	GGAGGGAGA	GCGGTCCC	CTGTCCCCAC	1910
AGTGGCATGA	CCCTCCGGTCT	CGCCAGGGT	GACAGGGGTG	TGACCGGGTC	GCTGTGAG
TCACCGTACT				CGACACGTC	CCACACGTC
1930	1940	1890	1950	1900	1910
TGTGCCTGGG	CCCCCTAGGG	TGCGGCTAG	CCAGGGGCTG	ACTGGCCCCAG	1920
ACACGGACCC	GGGGATCCC	ACCCCGAGTC	GGTCGGCGAC	TGACGGGGTC	GGTGGGAGT
				CGACACGTC	CCACACGTC
1990	2000	1950	2010	1960	1970
TTGCCAGGT	GGCCCTCCCT	CCAGCAGCAC	CTGCCCTGGG	CCCTCGGAGT	1980
AAACGGTCGA	CCGGJAGGGGA	GCTCGTCGTG	GACGGGACCC	CTGGGCCACG	GGAGGCC
				GGAGCCGTC	CCCTCGGAGT
2050	2060	2070	2070	2020	2030
GGAGCCCTG	GGGACAGACA	CACAGCCCT	GCCTCTGTAG	CTGACTGTTC	2040
CCTGGGGAC	CCCTGTCTGT	GTGTGGGGA	CGAGAACATC	CTCTGACAGG	TGTTCTGTGA
				ACAGAACACT	
2110	2120	2130	2130	2140	2150
GGGCCCTGT	CCCTCCGACC	TCCATGCCA	CTCGGGGCA	TGCTGGGAGT	2160
CGCGGGGACA	GGAGGGCTGG	AGGTACGGGT	GAAGCCCGT	ACGACCCCTA	GGGGTGAGCT
				CGCCACCCGA	
2170	2180	2190	2200	2150	2160
CTATGGCTTC	TGAGGCGAA	AGAACCCAGT	CTGGCTCTAG	TGCTGGGAGT	GGGGTGAGCT
GATAACGAG	ACTCCGCCCT	TCTGTGTGA	CCCCGAGATC	ACGACCCCTA	ACGGCTAGG
				CGCCACCCGA	
2230	2240	2250	2260	2210	2220
GTAGGGCGC	ATTAAGGCCB	GGGGGTGTG	GGGGCTCTAG	GGGGTATCCC	2230
CATGCCGGG	TAATTGCGGC	CGCCCAACACC	CCCCGAGATC	CGCCACCCGA	CACGGCC
				CGCCACCCGA	
2290	2300	2310	2320	2270	2280
CCAGGCCCT	AGCGCCCGCT	CCTTCGCTT	TCTTCCCTTC	TGTTCTGCCC	2340
GGTCGGGGGA	TCGGGGGGGA	GGAAAAGCGAA	ACCAATGCGC	GAGCTGAC	ACGTTGCCG
				CGATGTGAC	TGCAAGCGGC
2350	2360	2370	2380	2390	2400
GCTTCCCG	TCAAGCTCTA	AAATCGGGCA	TCCCTTCTAGG	GTTCCTGAGT	AGTGCCTTAC
CGAAAGGGGC	AGTTCGAGAT	TAGCCCGT	AGGGAAATCC	CAAGGCTAAA	TCACGMMATG

FIG. 19E

pD17-hG1b							
2410	2420	2430	2440	2450	2460		
GGCACCTCGA	CCCCAAAGAA	CTTGATTAGG	GTGATGTTTC	ACGTAGTGGG	CCATCGGCC		
CGGTGGGCT	GGGGTTTTT	GAACTAATCC	CACTACCAG	TGCAATCACC	GGTAGCCGA		
2470	2480	2490	2500	2510	2520		
GATAGACGGT	TTTCGCCCC	TTGACGTTGQ	AGTCCACGTT	CTTTAATAGT	GGACTCTGT		
CTATCTGCCA	AAAAGGGGA	AACTGCAACC	TCAGGTGCAA	GAATTATCA	CCTGAGAACAA		
2530	2540	2550	2560	2570	2580		
TCCAAGCTGG	AACAAACACTC	AACCCATCT	CGGTCTATTC	TTTGATTTA	TAAGGGATT		
AGGTTGACC	TTGTTGTGAG	TTGGGATAGA	GCCAGATAAG	AAAGCTAAAT	ATTCCCTAAA		
2590	2600	2610	2620	2630	2640		
TGGGATTTTC	GGCCTATTGG	TTAAAAAATG	AGCTGATTAA	ACAAAAAATT	AACGGGAATT		
ACCCCTTAAG	CCGGATAACC	AATTTTTAC	TGCACTAAAT	TGTTTTTAAA	TTGGCGCTTAA		
2650	2660	2670	2680	2690	2700		
AATTCTGTGG	AATGTGTATC	AGTTAGGGT	TGGAAAGTCC	CCAGGCTCCC	CAGGCAGGCA		
TTAACGACCC	TTACACACAG	TCAATCCAC	ACCTTTCAGG	GCTCCGAGGG	GTCCGTCCGT		
2710	2720	2730	2740	2750	2760		
GAAGTATGCA	AAGCATGCT	CTCAATTAGT	CAGCAACCAT	AGTCCCCCCC	CTAACTCCGC		
CTTCATAGCT	TTCGTACGTA	GAGTTAAATCA	GTCGTGGTA	TCAAGGGGGG	GATTGAGGCG		
2770	2780	2790	2800	2810	2820		
CCATCCGCC	CCTAACTCCG	CCCAGTTCCG	CCCAATTCTCC	CCCCCATGGC	TGACTAAATT		
GGTAGGGCGG	GGATTGAGGC	GGGTCAAGGC	GGGTAAAGGG	CGGGGTACCG	ACTGATTAAA		
2830	2840	2850	2860	2870	2880		
TTTTAATTA	TGCAAGAGGC	GAAGGCAGCT	CGGGCCTCTGA	GCTATTCCAG	AAGTAGTGA		
AAAAATAAT	ACGTCTCCGG	CTCCGGCGGA	GGCGGAGACT	CGATAAGTC	TTCAATCACTC		
2890	2900	2910	2920	2930	2940		
GAGGCTTTT	TGAGGGCTA	GGCTTTGGCA	AAAAGCTTGG	ACAGGCTCAGG	GCTGCCTATT		
CTCCGAAAAA	ACCTCCGGAT	CCGAAAGCT	TTTCGAAAC	TGTCGAGTCC	CGACGGCTAAA		
2950	2960	2970	2980	2990	3000		
CGCGCCAAC	TTGACGGCAA	TCCTAGCTGT	AAGGCTGGTA	GGATTATTC	CCCGCTGCCA		
GGCGCGTTG	AACTGCCGT	AGGATGCAAC	TTCCGACCAT	CCTAAATAG	GGGGGACGGT		

FIG. 19F

		pD17-hG1b	
3010	TCA TGG TCG ACC ATT GAC AGT ACC AAG C	3020 TGC AT CGTC G ACG TAG CAG C	3030 CCGT GT CCCA GGC AC AGGG T
3070	ACGG AGAC CT TGC CCT CGGA	3080 ACCG CT GGCT TGG GAC CGGA	3090 CCG CT CAGGA GGC GAG TCCT
3130	CAAC CT CTT C GTT GAG AAG	3140 AGT GGA AGGT TCAC CCT TCCA	3150 AAAC AGA ATC TTTG TCT TAG
3190	CCAT CCT GA GGT AAG GACT	3200 GAAGA AT CGA CTTC TTAGCT	3210 CCT TA AAGG GGA AATT CC
3250	TCA AAG AAC C AGTT CCT TGG	3260 ACC AC GAGGA TGG TGCT CCT	3270 GCT CATT TTTC CGAG TAA AAG
3310	TTATT GAA CA ATA ACT TGT	3320 ACCG GAA RTG TGG CCT TAA C	3330 GCA AGTA AAG CGTT CAT TTTC
3370	CTG TTACCA GACA AAT GGT	3380 GGA AGC CAT G CCT TCG GTAC	3390 AA TCA ACCA G TTAG TTGG TC
3430	TGC AGG AATT ACGT CCT TAA	3440 TGA AAGT GAC ACT TTCA CTG	3450 ACGTT TTTC TGCA AAA AGG
3490	TCC CAG AATA AGGG CCT TAT	3500 CCC AGG CGTC GGG TCC CGCAG	3510 CTCT CTG AGG GAG GAGCT CC
3550	TTG AAGT CTA AACT CAG AT	3560 CGAGA AGAAA GCT CCT CTT	3570 GACT AAC AGG CTG ATT GTCC

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FIG. 19G

		pD17-hG1b	
3610	3620	3630	3640
TAAAGCTATG	CATTTTATA	AQACCATGGG	ACTTTTGCTG
ATTTCGATAC	GTAAAAATAT	TCTGGTACCC	TGAAAACGAC
			GCTTTAGATC
			CGAAATCTAG
3670	3680	3690	3700
GGAACCTTAC	TTCTGTGGTGT	TGACATTAATT	GGACAAACTA
CCTTGGAAATG	AGAACACCAC	ACTGTATTAA	CCTACAGAGA
			GGATGTCTCT
			GGATGTCTGAG
3730	3740	3750	3760
TAAGGTTAAT	ATAAAATTTT	TAAGTGTATA	ATGTTTAA
ATTCATTTTA	TATTTTAAAA	ATTCACATAT	TACACAATT
			CTACTGATT
			GATGACTAAG
3790	3800	3810	3820
TGTATTTAG	ATTCACCAACTT	ATGGAACCTGA	TGAATGGGAG
ACATAAATC	TAACCTTGGAA	TACCTTGACT	ACTTACCCCTC
			GTCAACCACCT
			TACGGAAATT
3850	3860	3870	3880
TGAGGAAAC	CTCTTGTCT	CAGAAGAAAT	GCCATCTAGT
ACTCCTTTG	GACAAAACGAA	GTCTTCTTTA	CGGTAGATCA
			CTACTACTCC
			GATGACGACT
3910	3920	3930	3940
CTCTCAACAT	TCTACTCCCTC	CAAAAAAGAA	GAGAAAGGTA
GAAGGTGTA	AGATGTGGAG	GTTCCTTCCTT	CTCTGGGT
			TCCTGAAAGG
			AGGACTTCC
3970	3980	3990	4000
TTCAGAATTG	CTAAGTTTT	TAAGTCTTGC	TGTGTTTGT
AAAGTCTAAC	GATTCAAAAA	ACTCTGTACG	ACACAATTCA
			TTATCTTGAG
			AACGAACGAA
4030	4040	4050	4060
TGCTATTAC	ACCCACAAAGG	AAAAAGCTGC	ACTGCTATAC
ACGATAATG	TGGTGTTC	TTTTGCGACG	TGACGATATG
			TTCTTTTAAT
			ACCTTTTAT
4090	4100	4110	4120
TTCCTTAACC	TTTATAAGTA	GGCATATAACAG	TTATAATCAT
AAGACATTGG	AAATATTCAAT	CGCTATGTGTC	AACATACGT
			TTGTATGACA
			AAAAAGAATG
4150	4160	4170	4180
TCCACACAGG	CATAGAGTGT	CTCTTAA	TAACTATGCT
AGGTGTGTC	GTATCTCACCA	GCACATAATT	CAAAATCTG
			TTTTTCTTAC
			GTACCTTAG
			CATGQAATC

FIG. 19H

		pD17-hG1b	
4210	4220	4230	4240
CTTTTAATT	TGTAAGGGG	TTAATAAGGA	ATATTGTAG
GAATAATTAA	ACATTCCCCC	AATTATTCCCT	TATAAACTAC
4270	4280	4290	4300
TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC
AGTATTAGTC	GGTATGGTGT	AAACATCTCC	AAAATGAACG
4330	4340	4350	4360
TCCCCCTGAA	CCTGAAACAT	AAAATGAATG	CAATTGGTGT
AGGGGGACTT	GGACTTTGTA	TTTACTTAC	GTTAACACAA
4390	4400	4410	4420
CTTATAATGG	TTACAAATAA	AGCAAATAGCA	TCACAAATT
GAATATTACCC	AATGTTTTATT	TGTTTATCGT	AGTGTTTAAA
4450	4460	4470	4480
CACTGCATC	TAGTGTGGT	TGTCACAAAC	TCATCAATGT
GTGACGTAAG	ATCAACACCA	AAACGGTTTG	AGTAGTTACA
4510	4520	4530	4540
GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTF
CGACCTACTA	GGAGGTGCGC	CCCCTAGAGT	ACGACCTCAA
4570	4580	4590	4600
TTATTCAGC	TTATAATGGT	TACAAATAAA	GCATAAGCAT
AATAACGTG	AATATTACCA	ATGTTTATT	CGTTATCGTA
4630	4640	4650	4660
CATTTTTC	ACTGCATTCT	AGTTGGGGTT	TGTCACAAACT
GTAAAAAAAG	TGACGTAAGA	TCAACACCAA	ACAGTTTGA
4690	4700	4710	4720
TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC
AGACATATGG	CAGCTGGAGA	TCGATCTCGA	ACCGCATTAG
4750	4760	4770	4780
TGTGAATATG	TTATCCGGCTC	ACATTCCAC	ACAAACATAG
ACACTTTAAC	AATAGGCCGAG	TGTTAAAGGTG	TGTTGTATGC

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pD17-hG1b							
4810	4820	4830	4840	4850	TGGCTTGGC		4860
AAGCCCTGGG TTCGAACCC	TGCCATAATGA ACGGATTACT	GTGAGGTAAC CACTCGATTG	TCACTTAAT AGTGTAAATT	ACGCAACCGG		TCACTGGCCC AGTGACGGGC	
4870	4880	4890	4900	4910	AATCGGCCAA TTAGCCGGTT		4920
CCTTCCAGTC GAAAGGTAG	GGGAAAACCTG CCCTTGGAC	TGGTGCAGC ACGACGGTGC	TGGCTTAATG ACGTAATTAC		CGCGGGGAA GCGGCCCT		
4930	4940	4950	4960	4970	CACTGACTCG GTGACTGAGC		4980
GAGGGGGTTT CTCGGCCAA	GCGTATGGG CGGATAACCC	CGCTCTTCGG GCGAGAAGGC	CTTCTCGCT GAAGGAGGGA		CTGGCTCGG GACGGAGCC		
4990	5000	5010	5020	5030	GGTMAATACGG CCATTATGCC		5040
TCGTTGGCT AGCAAGCCGA	CGGGCGGCC3 CGCGCGCTGC	GTATCGCTC CATAGTCGAG	ACTCAAAGGC TGAGTTCCG		TTATCCACAG AATAGGTGTC		
5050	5060	5070	5080	5090	CCAGCAAAG GGTCGTTTC		5100
AATCAGGGGA TTAGTCCCT	TAACGAGGA ATTGCGTCT	AAGAACATGT TTCTTGATCA	GGGCAAAGG CTCGTTTC		GCCAGGAACC CGGTCTTGG		
5110	5120	5130	5140	5150	CCCCCTTGAC GGGGGGACTG		5160
GTAAGGGC CATTTTCGG	CGGGTTGCTG GGCCAACGAC	GGGTTTTTC CGCAAAAGG	ATAGGCTCCG TATCCGAGGC		GAGCATCACA CTCGTAGTGT		
5170	5180	5190	5200	5210	ACTATAAGA TGTATTTCT		5220
AAAATCGACG TTTTAGCTG	CTCAAGTCAG GAGTCAGTC	AGGTGGGAA TCCACCGCTT	ACCCCAACGG TGGGCTCTCC		TACAGGGCGT ATGTCGGAA		
5230	5240	5250	5260	5270	GGACAGGCTG CCTGCCGGTT		5280
TTCCCCCTGG AAGGGGACCC	AAGCTCCTC TTCGAGGGAG	GTGCGCTCTC CAGCGGAGAG	CTGTCGAC GACAAGGCTG		ACGGATAACC TGGCTATGG		
5290	5300	5310	5320	5330	GGAAAGCGTG CCTTCGCACC		5340
TGTCCGGCTT ACAGGGGAA	TCTCCCTTCG AGAGGGAAAC	CGAAGCGCTG CCTTCGCACC	CGCTTCTCA GCGAAAGAGT		ATGGCTCACGC TACGAGTGG		
5350	5360	5370	5380	5390	GCACGAAACCC ACCCGACACA		5400
TCAATTCTGG AGTCAGGCCA	GTAGGTCTTT CATCCAGCAA	CGCTTCAAGC GGGAGGGTTCG	TGGGCTTGTGT ACATCCATAG		CCGGTTCAAGC GGGCAAGTGGG		

FIG. 19 J

pD17-hG1b						
5410	5420	5430	5440	5450	5460	
CCGACCGCTG	CGCCCTTATCC	GGTAACATTC	GTCCTGAGTC	CAACCCGGTA	AGACACGACT	
GGCTGGGAC	GGCAAATAGG	CCATTGATAG	CAGAACTCAG	GTGGGCCAT	TCTGTGCTGA	
5470	5480	5490	5500	5510	5520	
TATGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	ACCGAGGTAT	GTTAGGGCGGTG	
ATAGCGGTGA	CCGTCTGGG	TGACCATGTT	CCTATCGTC	TCGCTTCATA	CATCCGCCAC	
5530	5540	5550	5560	5570	5580	
CTACAGAGTT	CTTGAACTG	TGGCCTAATC	ACGGCTACAC	TAGAAAGACA	GTATTGGTA	
GATGCTCACC	GAACCTCACC	ACCGGATGTA	TGCCGATGTC	ATCTTCCTGT	CATAAACCAT	
5590	5600	5610	5620	5630	5640	
TCTGCGCTT	GCTGAAGCCA	GTTACCTCG	GAATAAGAGT	TGGTAGCTCT	TGATCCGGCA	
AGACGGGAGA	CGACTTCGGT	CAATGGAAGC	CTTTTCTCA	ACCATCGAGA	ACTAGGCCGT	
5650	5660	5670	5680	5690	5700	
AACAAACAC	CGCTGGTAGC	GGTGGTTT	TIGTTTGCAA	GCAGGAGATT	ACGCCAGAA	
TGTTGGTG	GGCACCATCG	CCACCAAAAA	AAACAAACGTT	CCTCGCTAA	TGGCGGTCTT	
5710	5720	5730	5740	5750	5760	
AAAAGGATC	TCAAGGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCAGACGCT	CGTGTGAAACG	
TTTTCTCTAG	AGTTCTCTA	GGAAACTAGA	AAAGATTCGG	CAGACTGCGA	GTCAACCTTCG	
5770	5780	5790	5800	5810	5820	
AAACTCACG	TTAAGGGATT	TTGGTCATGA	GATTATCAA	AAGGATCTTC	ACCTAGATCC	
TTTTGAGTGC	AATTCCCTAA	AACCAAGTACT	CTAAATAGTT	TTCCCTAGAAG	TGATCTAGG	
5830	5840	5850	5860	5870	5880	
TTTTAAATA	AAAATGAAGT	TTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	
AAATTTTAA	TTTTACTTCA	AAATTTAGT	AGATTTCATA	TATACTCATT	TGATCCAGAC	
5890	5900	5910	5920	5930	5940	
ACAGTTACCA	ATGCTTAATC	AGTGGGGAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTCTAT	
TGTCAATGGT	TACGAATTAG	TCACTCCGTG	GATAGAGTCG	CTAGACAGAT	AAAGCAAGTA	
5950	5960	5970	5980	5990	6000	
CCATAGTTGC	CTGACTCCCC	GTCGTGAGA	TAATACGAT	ACGGAGGGC	TTACCATCTG	
GGTATCAGC	GACTGAGGGG	CAGCACCTCT	ATTGATGCTA	TCCCCTCCG	ATGGTAGAC	

FIG. 19K

pD17-hG1b					
6010	6020	6030	6040	6050	6060
CCCCAGTGC	TGCAATGATA	CCGGAGACC	CACGCCAAC	GGCTCCAGAT	TTATCGCMA
CGGGTCACG	ACGGTACTAT	GGCGCTCTGG	GTGCGAGTGG	CGAAGGATCTA	AATAGTCGTT
6070	6080	6090	6100	6110	6120
TAAACAGGCC	AGCCGGAAAGG	GCCGAGGGCA	GAAGTGGTCC	TGCAACTTCA	TCCGCCTCCA
ATTGGTCGG	TGGGCCCTTCC	CGGCTCGCGT	CTTCACCAAGG	ACGTTGAAT	AGGGGGAGGT
6130	6140	6150	6160	6170	6180
TCCAGTCTAT	TAATGGTGC	CGGGAAGCTA	GAGTAAGTAG	TTCGCCAGAT	AATAGTTTGC
AGGTCAATA	ATTAACACCG	GCCCTTCGAT	CTCATTCATC	AAGCGGTCAA	TTATCAAACG
6190	6200	6210	6220	6230	6240
GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGCTGTCACG	CTCGTCTGRTT	GGTATGGCTT
CGTTGCAACA	ACGGTAACGA	TGTCCGTAGC	ACCACAGTGC	GAGCAGGCAAA	CCATACCGAA
6250	6260	6270	6280	6290	6300
CATTAGCTC	CGGTTCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGGCAMA
GTAAGTCGAG	GCCAAGGGTT	GCTAGTTCCG	CTCAATGTAC	TAGGGGTAC	AACACCTTT
6310	6320	6330	6340	6350	6360
AAGGGTTAG	CTCCCTCGGT	CCTCCGATCG	TTCCTGAAAG	TAAGTTGGCC	GCAGTCCTAT
TTGCCATC	GAGGAAGCCA	GGAGGCTAGC	AAACAGTCTTC	ATTCAACCGG	CCTCACAAATA
6370	6380	6390	6400	6410	6420
CACTATGGT	TATGGCAGCA	CTGCATAATT	CTCTCTACTGT	CATGCCATTC	GTAAGATGCT
GTGAGTACCA	ATACCGTGT	GACGTATTAA	GAGAATGACA	GTACGGTAGG	CATTCTACGA
6430	6440	6450	6460	6470	6480
TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCCACCGA
AAAGACACTG	ACCACTCTG	AGTGGTTCA	GTAAGACTCT	TATCACATAC	GCCGGCTGGCT
6490	6500	6510	6520	6530	6540
GTTGCTCTTG	CCCGGGGTCA	ATACGGGATA	ATACCGGCC	ACATAGCAGA	ACTTTAAAG
CAACGAGAAC	GGGGCGCAGT	TATGCCCTAT	TATGGCGGG	TGTATCGTCT	TGAAATTTC
6550	6560	6570	6580	6590	6600
TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GGAAACTCTC	AGGAACTCTA	CCACTCTGAA
ACGAGTAGTA	ACCTTTGCA	AGAAGCCCCG	CTTTTGAGAG	TTCCTAGAAT	GCCGACCACT

FIG. 19L

pD17-hG1b							
6610	6620	ACTCGTGCAC	6630	CCAAGTCATC	6640	TTCAAGCATCT	6650
GATCCAGTTTC	GATGTAACCC	TGAGCACGGT	GGTGTGACTG	AAGTCGTAGA	TTTACTTCA	AAATGAAGT	6660
CTAGGTCAAAG	CTACATTGGG						
6670	6680	AAAACAGGAA	6690	GGCAAAATGC	6700	CGCAAAAAAG	6710
CCAGCGTTTC	TGGGTAGCA	TTTTTCTCCCT	CCGGTTTTCG	CCGGTTTTTC	CCGGTTTTTC	GGATAAGGG	6720
GOTCGCAAG	ACCCACTCTGT						
6730	6740	CTCATACTCT	6750	TCCTTTCTCA	6760	ATATTATTGA	6770
CGACACGGAA	ATGTTGAATA	GAATGTGAGA	AGGAAAGT	TATAATAACT	AGCATTATC	TCGTAATAATG	6780
GCTGTGCCCT	TACAACCTAT						
6790	6800	GGATACATAT	6810	TTGAATGTAT	6820	TTGAAAMAT	6830
ACGGTTATTG	TCTCATGAGC	CCTATGTATA	AACTTACATA	AATTTTTTA	AAACAAATAG	TTTGTTTATC	6840
TCCCAATAAC	AGAGTACTCG						
6850	6860	CGAAAGTGC	6870	CACCTGACGT	6880	CGACGGATCG	6890
GGGTTCCGGG	CACCTTCCC	GCTTTCAGG	GTGGACTGCA	GCTGCCCTAGC	GCTCTAGACG	GGAGATCTGC	6900
CCCAAGGGCC	GTGTAAGGG						
6910	6920	GGCTTCAAT	6930	AGCCAGAGTA	6940	6950	6960
TAGTGTACCT	GAAGGCGCGC	CCGAAGCTTA	TCGGTCTCAT	TGGAAAAAA	ACCTTTTT	TTAATTTTAT	
ATCCACTGGA	CTCCCGGGGG						
6970	6980	GGGTTGGG	6990	CCGATCTCCC	7000	7010	7020
TTTATTAT	TTTGTGAGTG	GAGTTGGG	CGCTAGGGG	GATCCCTAT	GACGAGGGAA	GGTCGACTCT	
AAATAAAATA	AAAACTCTAC	CTCAAAACGGC				CCAGCTGAGA	
7030	7040	CCGGCATAGTT	7050	AAGCCAGTAT	7060	7070	7080
CACTACAAATC	TGCTCTGATG	GGCGTATCAA	TTCGTGTATA			CTGCTCCCTG	
GTCATGTAG	ACGAGACTAC					CTGTGTGTT	
7090	7100	CGAGCAAAT	7110	TTAACGCTACA	7120	7130	7140
GGGGGTGGCT	GAGTAGTGGG	GCTCGTTTA	AATTGATGT			GCTTGACCGA	
CCTCCAGGGA	CTCATCACGC					CGAAACTGGCT	
7150	7160	TTAGGGTGTAG	7170	GCCTTTTGC	7180	7190	7200
CAATTGATG	AGAATCTGC	AATCCCAATC	CCCAAAACGC	CGCAAAAGGC	CGCAAAACGC	ATGTACGGGC	
GTTAACGTAC	TTCCTAGACG					TACATCCCC	

FIG. 19M

pD17-hG1b					
7210	7220	GAATTGAC	7230	7240	7250
CAGATATA	CGTGACATT	GATTATGAC	TAGTTATAA	TAGTAAATCAA	TTACGGGTCA
GTCTATATGC	GCAACTGAA	CTAATACTG	ATCATAATT	ATCATTAAGTT	AATGCCCAAG
7270	7280	TGGAGTCCG	7290	7300	7310
ATTAGTCAT	AGCCCATA	CGTTACATAA	CTTACGGTAA	ATGGCCGCC	ATGGCCGCC
TAATCAAGTA	TCGGGTAT	GCATGTATT	GAATGCCATT	TACCGGGCGG	
7330	7340	CCCGCCATT	7350	7360	7370
TGGCTGACCG	CCCAACGACC	GACGTCAATA	ATGACGTATG	TTCCCATAGT	
ACCGACTGCC	GGGTTGCTGG	CTGCAAGTT	TACTGCATAC	AAGGGTATCA	
7390	7400	ATGACGTCA	7410	7420	7430
AACGCCATA	GGGACTTCC	ATGGGTGGAC	TATTACGGT	AAACTGCCA	
TTGGGGTTAT	CCCTGAAAGG	TAACTGCACT	TACCCACCTG	TTTGACGGT	
7450	7460	ATCATATGCC	7470	7480	7490
CTTGACAGTA	CATCAAGTGT	AGTACGGCC	CCTATGACG	TCAATGACGG	
GAACCGTCAT	GTAGTTCA	TAGTATACGG	GGATAACTGC	AGTTACTGCC	
7510	7520	ATGCCAGTA	7530	7540	7550
TAATGGCC	GCCTGGCATT	CATGACCTTA	TGEGACCTTC	CTRACTTGGCA	
ATTACGGG	CGAACCGTA	GTACTGGCAT	ACCTGAAAG	GATGAAACCGT	
7570	7580	TCGCTTAC	7590	7600	7610
GTACATCTAC	GTATTAGTC	CATGGTGTG	CGGTGTTGGC	ACTACATCAA	
CATATAGTG	CATAATCAGT	AGCGATAATG	GCCAAACCG	TCATGTAGTT	
7630	7640	ACTCACGGG	7650	7660	7670
TGGGGCTGGA	TAGGGTTTG	TTTACTGCCCC	CTCCACCCA	TTGACGTCAA	
ACCCGCACT	ATGCCCAAAAC	TAAAGGTCA	GAGGTGGGT	AACTGGAGTT	
7690	7700	AAAATCAACG	7710	7720	7730
ACCCCTAAAC	AAAACCGTGG	TTTACTGTC	AAATGTGTA	ACAACTCGC	
7750	7760	GTAGGGCG	7770	7780	7790
CCCATGGACG	CAAAATGGCG	ACGGTGGAG	GTCTATATAA	GCAGAGGCTCT	
GGGTAACTGC	GTTCACCCGC	CATCCGCACA	TGCCACCCCTC	CAGATATATT	CGTCTCGAGA

FIG. 19N**pD17-hG1b**

CTGGCTTAAC	7810	AGAGAAACCA	7820	CTGCTTACTG	7830	GCTTATCGAA	7840	ATTAATAACGA	7850	CTCACTATAG	7860
GACCGATTGA		TCTCTGGGT		GACGAATGAC		CGAATAGCTT		TAATTATGCT		GAGTGATATC	
	7870				7880						
		GGAGACCCAA		GCTT							
		CCTCTGGGT		CGAA							

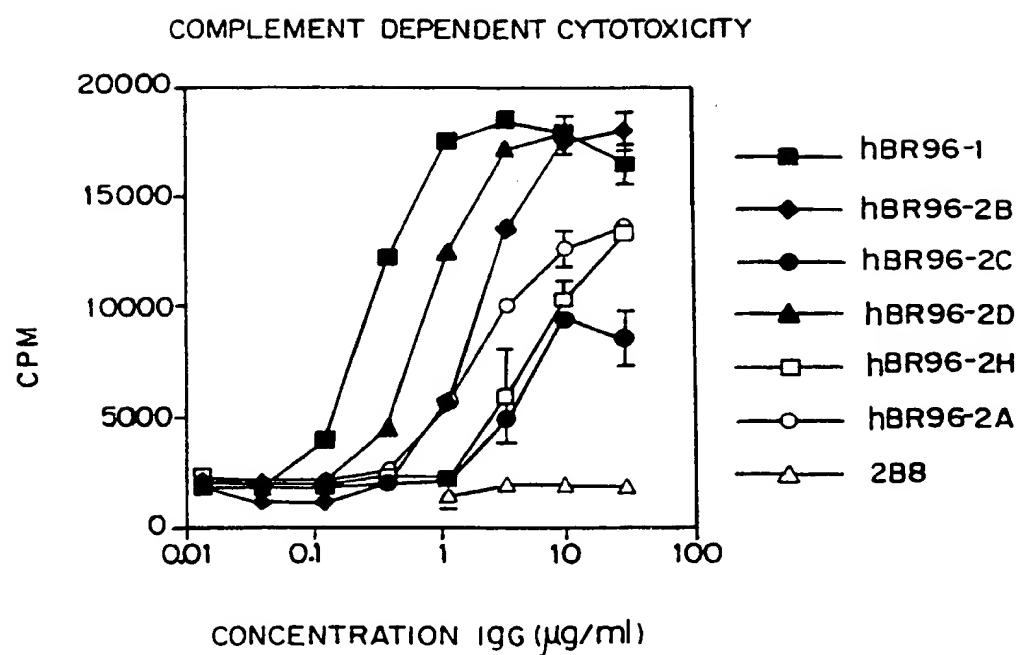
Fig. 20

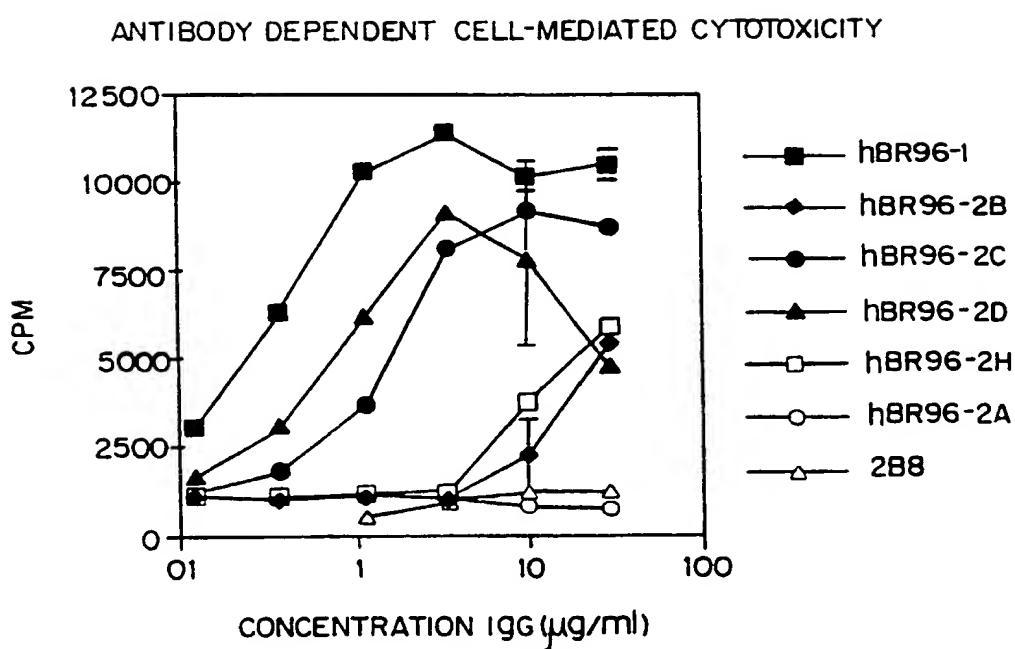
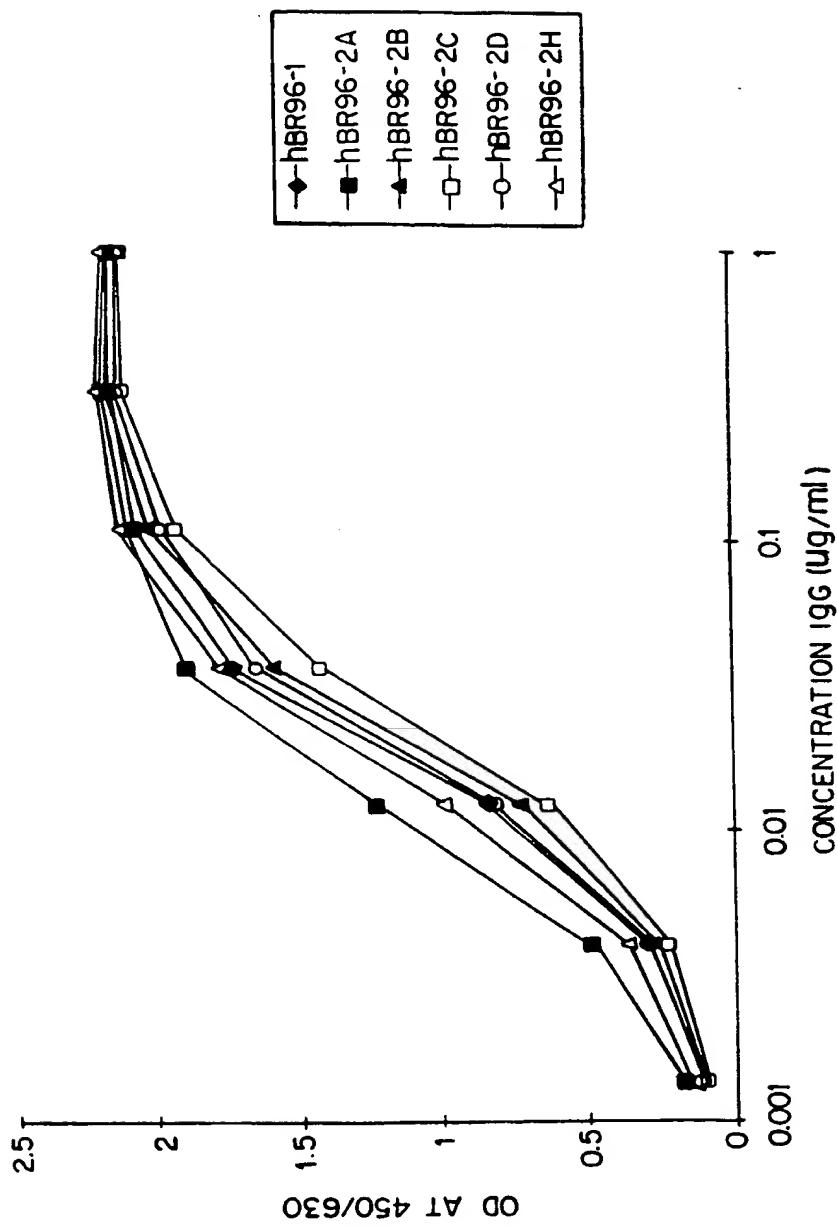
Fig. 21

Fig. 22

BINDING ACTIVITY OF hBR96-2 CONSTANT REGION MUTANTS ON LEY-HSA



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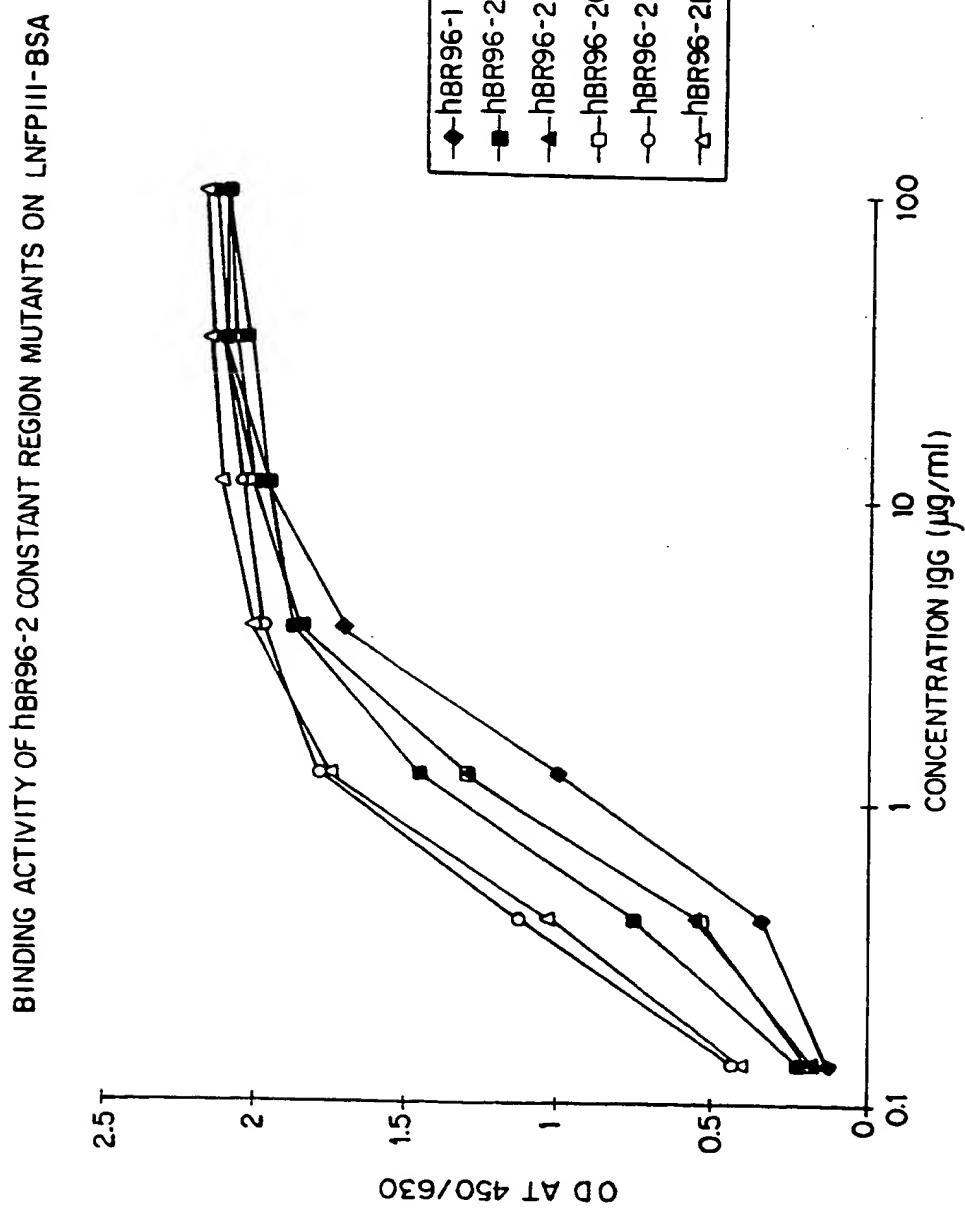
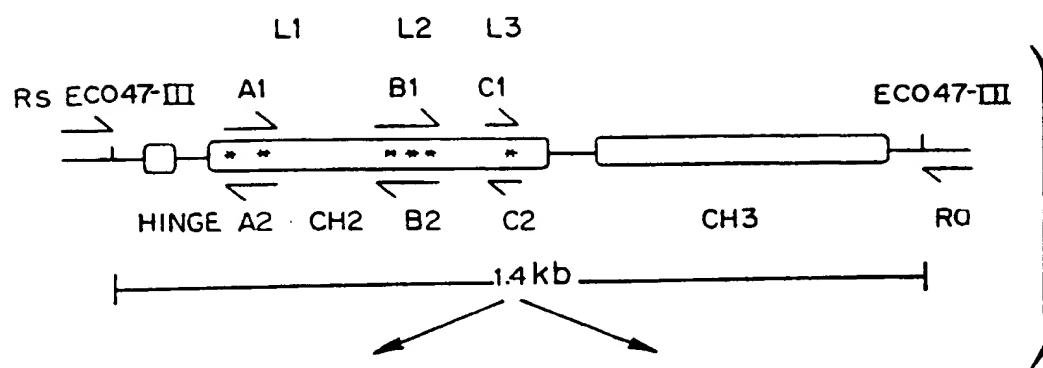
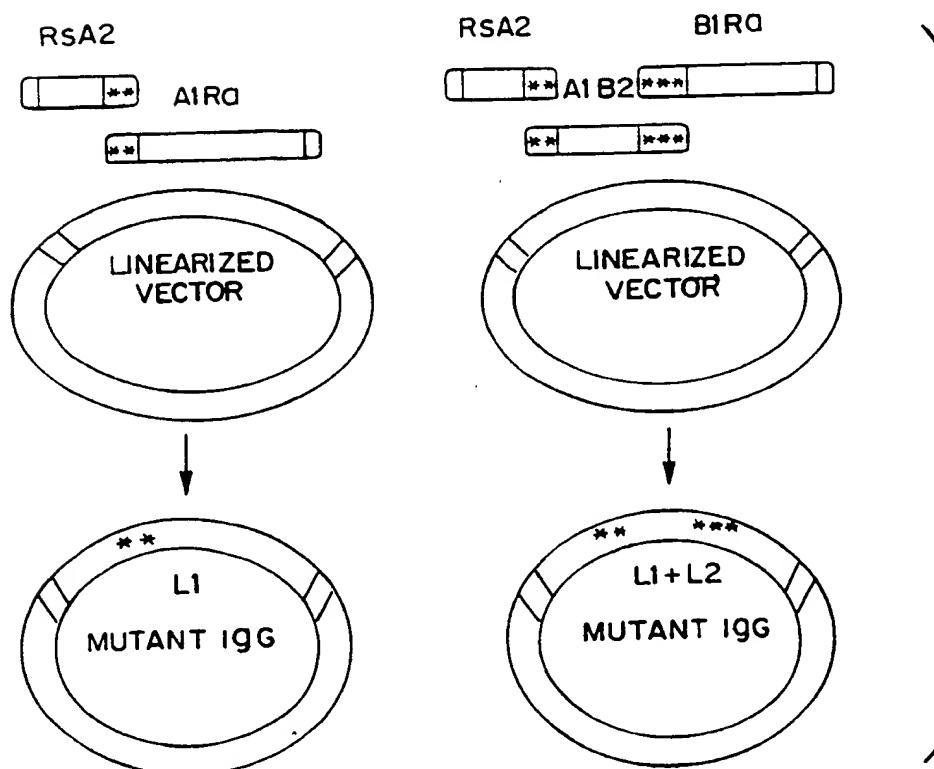
Fig. 23

Fig. 24A***Fig. 24B***

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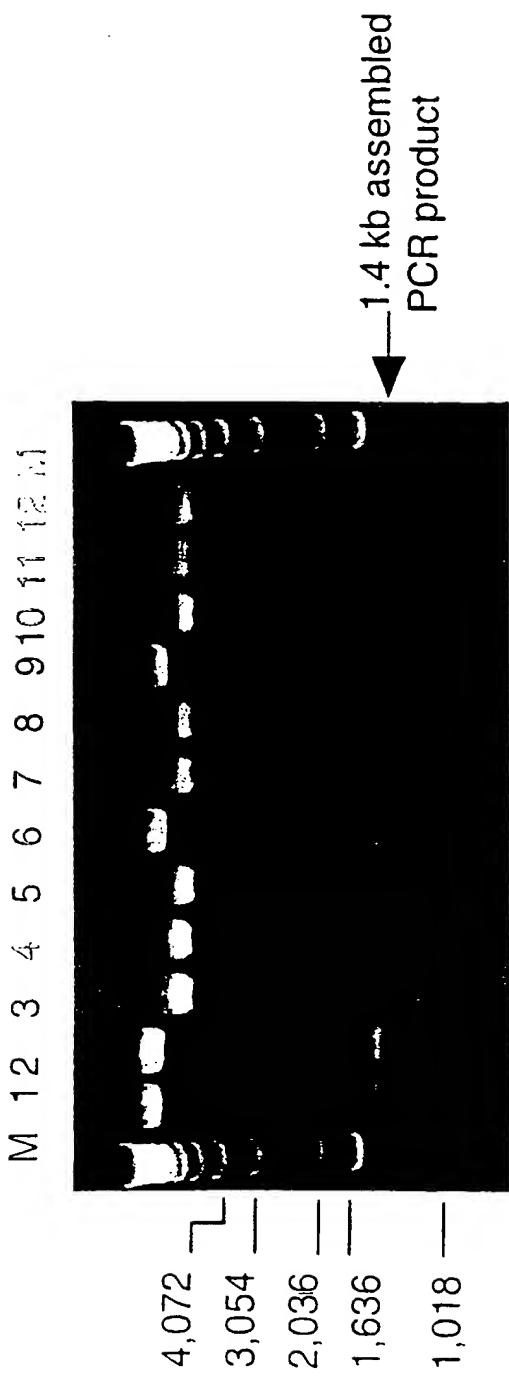


FIG. 25

FIG. 26

hBR96-2 Heavy Chain Variable Region (V_H)

1 11 21 31 41
 EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYYMYWVRQA PGKGLEWVSY
 51 61 71 81 91
 ISQDGDDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
 101 111
 ADGAWFAYWG QGTLVTVSS

Human IgG1 Constant

CH1
A STKGPSVFPL APSSKSTSGG TAALGCLVKD

YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CHAPELLGGP SVFLFPPKPK
DTLMISRTP E VTCVVVDVSH EDPEVKFNWY VDGVEVHNAA TKPREEQYNS
TYRVVSVLTV LHQDWLNGKE YKDVKVSNKAL PAPIEKTISK AKGQPREPV
YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPV
DSDGSFFLYS KLTVDKSRWO OGNVFSCSVM HEALHNHYTQ KSLSLSPGK

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FIG. 27**hBR96-2A: Heavy Chain Variable Region (V_H)**

1 11 21 31 41
EVQLVESGGG LVQPGGSLRL SCAASGFPPFS DYYMYWVRQA PGKGLEWVSY
51 61 71 81 91
ISQDGDDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
101 111
ADGAWFAYWG QGTLTVSS

hBR96-2A: Human Heavy Chain IgGl Constant Region ΔCH2

A STKGPSVFPL APSSKSTSGG TAALGCLVKD YFPEPVTVSW NSGALTSGVH
TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY ICNVNHKPSN TKVDKKVEPK
SCDKTHTCPP CP GQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA
VEWESNGQPE NNYKTPPPVLDSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM
HEALHNHYTQ KSLSLSPGK

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FIG. 28

This sequence is the chi BR96 IgG1 with CH2 deleted.

VH
1 EVNLVESGGG LVQPGGSLKV SCVTSGFTFS DYYMYWVRQT PEKRLEWVAY
51 ISQGGDITDY PDTVKGRFTI SRDNAKNTLY LQMSRLKSED TAMYYCARGL
CH1
101 DDGAWFAYWG QGTLTVVSVA STKGPSVFPL APSSKSTSGG TAALGCLVKD
151 YFPEPVTVSW NSGALTSGVH TFPAVLQSSG LYSLSSVVTV PSSSLGTQTY
CH3
201 ICNVNHKPSN TKVDKKVEPK SCDKTHTCPP CPGQPREPQV YTLPPSRDEL
251 TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTPPPVL DSDGSFFLYS
301 KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK

INTERNATIONAL SEARCH REPORT

al Application No

PCT/US 97/13562

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6	C12N15/62	A61K39/395	A61K38/17	A61K47/48	A61K51/10
	C07K16/30	C07K16/46	C07K16/00	C12N15/13	C12N1/21
	C12N5/10	//C07K19/00			

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. GILLIES ET AL.: "Antigen binding and biological activities of engineered mutant chimeric antibodies with human tumor specificities." HUMAN ANTIBODIES AND HYBRIDOMAS, vol. 1, no. 1, 1990, STONEHAM, MA, USA, pages 47-54, XP002050448 see the whole document --- -/-	1-8, 23-25



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

1

Date of the actual completion of the international search

17 December 1997

Date of mailing of the international search report

21.01.98

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Fax: (+31-70) 340-3016

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Nooij, F

INTERNATIONAL SEARCH REPORT

PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No..
X	G. SCHREIBER ET AL.: "An unmodified anticarcinoma antibody, BR96, localizes to and inhibits the outgrowth of human tumors in nude mice." CANCER RESEARCH, vol. 52, no. 12, 15 June 1992, BALTIMORE, MD, USA, pages 3262-3266, XP002050449 see abstract	33,35,36
A	---	1,2,5,7, 8,11-18, 23
A	A. DUNCAN ET AL.: "The binding site for C1q on IgG." NATURE, vol. 332, no. 6166, 21 April 1988, LONDON, GB, pages 738-740, XP002050450 cited in the application see the whole document	1,2,5,7, 8
A	J. LUND ET AL.: "Human FcgammaRI and FcgammaRII interact with distinct but overlapping sites on human IgG." THE JOURNAL OF IMMUNOLOGY, vol. 147, no. 8, 15 October 1991, BALTIMORE, MD, USA, pages 2657-2662, XP002050451 cited in the application see abstract	1,2,5,7, 8
A	---	1-8
A	Y. XU ET AL.: "Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 5, 4 February 1994, BALTIMORE, MD, USA, pages 3469-3474, XP002050452 cited in the application see abstract see discussion	
A	---	1,2,5,7, 8
1	T. MICHAELSEN ET AL.: "One disulfide bond in front of the second heavy chain constant region is necessary and sufficient for effector functions of human IgG3 without a genetic hinge." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 20, 27 September 1994, WASHINGTON, DC, USA, pages 9243-9247, XP002050453 see the whole document	

	-/-	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation or document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	L. TAN ET AL.: "Influence of the hinge region on complement activation, Clq binding, and segmental flexibility in chimeric human immunoglobulins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 87, no. 1, January 1990, WASHINGTON, DC, USA, pages 162-166, XP002050454 see the whole document ---	1-8
A	EP 0 699 756 A (BRISTOL-MYERS SQUIBB COMPANY) 6 March 1996 cited in the application see examples see claims -----	11-18, 23,25, 28,29, 31-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/13562

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/13562

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 26,27

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 26 represents a method of detection/diagnosis and refers forward to claim 30, which represents a method of treatment. Claim 27 refers to a method in claim 24; however, in claim 24 a product is claimed, not a method.

Remark : Although claims 1-22, 25, 28-32 and 34-36 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 97/13562

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 699756 A	06-03-96	AU 2834995 A CA 2155397 A JP 8191692 A	15-02-96 05-02-96 30-07-96

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